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**Laboratory and Field Studies of Insecticide Impregnated Fibres
for Mosquito Control**

by

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in the University of London**

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NUMEROUS ORIGINALS IN COLOUR



ABSTRACT

Laboratory and field studies were undertaken in an attempt to improve mosquito control with insecticide-treated fabrics. The efficacies of the emulsifiable concentrates (E.C.) of three pyrethroids were assessed on three types of fibre in order to establish the optimum combination for bednet use. Various pyrethroid formulations were compared for persistence and wash-fastness. Incorporation of polystyrene into the E.C. of permethrin increased its wash-resistance.

Scanning electron microscope studies revealed a film of permethrin on impregnated nylon fibre which was not visible on similarly impregnated cotton fibre.

Using an acoustic actograph to record flight activity and video to record behaviour, impregnated nylon was found to cause greater irritancy than impregnated cotton. The contact times for mosquitoes to pick-up lethal doses from permethrin and lambdacyhalothrin treated netting were established and related to the time that a hungry mosquito spends searching for a bloodmeal.

Studies on sublethal effects of treated netting such as knock-down, leg fracture and feeding inhibition were carried out.

Experimental hut trials of bednets were undertaken in The Gambia to compare five different insecticides, an insecticide mixture and two formulations incorporating polystyrene. The unwashed permethrin formulations strongly deterred hut entry by An.gambiae s.l. and Mansonia spp. but this effect was lost after washing the nets three times. The unwashed insecticide-treated nets killed a significantly higher percentage of An.gambiae s.l. that entered the huts than the untreated net. Washing treated nets decreased the percentages killed by them, with the exception of the wash-resistant permethrin which showed no significant change in the percentage of mosquitoes killed. This formulation killed a significantly higher percentage than the normal permethrin formulation after washing. The unwashed mixture of permethrin and pirimiphos-methyl treated net performed well over the 6 weeks of the trial, but chemical analyses at the end of this time showed very little of its pirimiphos-methyl remained on the net. The effects of all the nets on numbers entering and exiting from

huts and numbers found killed and bloodfed are reported.

A trial was carried out in a village to compare nets treated with the wash-resistant formulation of permethrin, normal E.C.s of permethrin and lambdacyhalothrin and placebo. The numbers of mosquitoes found over 16 weeks during weekly bednet searches of the insecticide treated nets were greatly reduced compared with the placebo treated nets. Analysis of bioassay mortality showed that the permethrin formulation containing polystyrene (wash-resistant) was significantly less affected by washing under village conditions than the normal permethrin and lambdacyhalothrin formulations.

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CHAPTER 1

INTRODUCTION

1.1 General Introduction.

Malaria still kills. Two recent estimates put the annual child mortality due to malaria at 1.9 and 0.5 million in Africa alone (Stürchler, 1989; Greenwood, 1989). Millions of people all over the tropical world are debilitated by this destructive disease and the socio-economic development of many countries is impeded by it. Millions more would simply like to sleep at night undisturbed by mosquitoes. Mosquito bednets impregnated with insecticides can alleviate the problem of nuisance mosquitoes and may control and even help to eradicate malaria in some areas of the world.

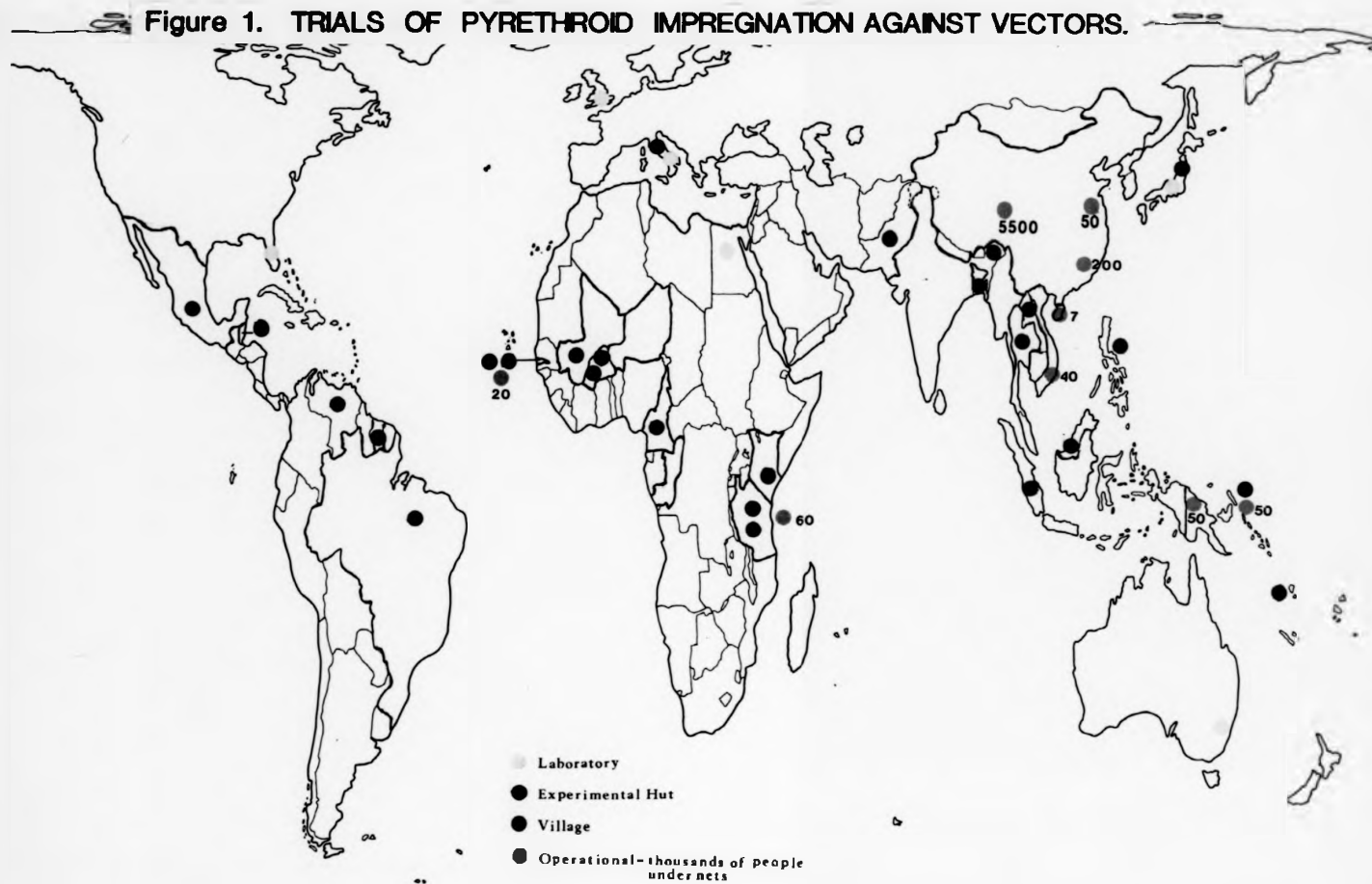
The use of pyrethroid impregnated bednets has gathered pace in a remarkable fashion over the last five years. There have been, or are, studies being carried out in 30 countries world wide (Figure 1) and the list seems to be increasing steadily. There are many reasons for this - the simplest being that impregnated bednets kill mosquitoes or deter them from entering houses.

Impregnation of nets could theoretically reduce malaria transmission in two ways: by

- (i) decreasing the vectorial capacity of a mosquito population as a whole through an increase in mosquito mortality;
- (ii) by providing protection to individuals sleeping under treated nets (or, to some extent, in houses when other occupants have treated nets) by repellency or irritancy, or through inhibition of biting (Graves *et al.*, 1987).

Bednets are easy to impregnate, they are taken down, dipped in an emulsion of insecticide and water, wrung out (or allowed to drip dry in the case of some synthetics) and left to dry. This takes a few minutes. No furniture needs to be removed, there is no risk of food becoming contaminated, there are no

Figure 1. TRIALS OF PYRETHROID IMPREGNATION AGAINST VECTORS.



malodorous or powdery residues (although the net may be a little pungent for a day or so, this soon wears off), and no spray equipment or specially trained personnel are needed. The increasing move towards primary health care and allowing people to determine their own objectives in the control of diseases and household nuisances is very favourable to the concept of impregnated nets.

Impregnation also has beneficial side effects e.g. the reduction of headlice and bedbugs as reported by several workers (see section 1.11).

Impregnation of bednets is probably cheaper than wall spraying. This is certainly true where the population already has nets. For example, in an area of China where most of the population have nets, Li Zuzi has compared the cost of a residual house spraying programme using DDT with the impregnation of bednets using deltamethrin and found the cost to be \$0.12 and \$0.065 per person, per annum respectively (data in Curtis *et al.*, 1989). This follows partly from the much higher dosage of DDT than deltamethrin used per unit area and also from the fact that the area of wall to be sprayed in a typical Chinese house is three times the area of the nets to be impregnated. Thus the use of insecticide treated bednets is a focused intervention which requires far less insecticide than spraying the walls and ceiling of the house containing them (MacCormack *et al.*, 1989).

Impregnated bednets may also be more effective than house spraying - especially in cases where the endophagic but exophilic behaviour of the vectors is a major drawback to effective use of house spraying. Resistance (both physiological and behavioural) to conventional insecticides used in house spraying, e.g. DDT, combined with difficulties in organizing and paying for large scale spraying programmes and the increasing non-cooperation of householders with house spraying created an ideal spring board for the launching of impregnated bednets.

1.2 Bednet Use World Wide.

All this presupposes that people either use nets already or will easily adapt to using them. The extent to which bednets are used world-wide is very difficult to estimate. In some countries usage is very high, for example, MacCormack and Snow (1986) report that in The Gambia as many as 99% of rural Mandinka people sleep under bednets, but only 64% of Wolof and 58% of Fulas do so. In Suriname, 95% of Ndjuka ("bushnegro") and 100% of Amerindians use them (Rozendaal *et al.*, 1989). A study carried out in six villages in northern Thailand found that although 81.8% of households in the villages owned mosquito nets and 69.7% of villagers working outside the villages carried them to the forest, their actual use was somewhat irregular. When there was a low density of mosquitoes some people did not consider it worthwhile to sleep under a net (Chitprarop *et al.*, 1986). Ogbalu (1980), who interviewed 211 people in Nigeria found that about 57% were not using bednets because of cost or inconvenience.

In a survey conducted in Tanzania by White (1969) only 7% of dwellings inspected had sufficient bednets for all sleepers. Bednets in Tanzania are very expensive on the retail market, costing the equivalent of one month's minimum wage (Curtis *et al.*, 1989) and consequently bednet use is still very low.

1.3 Untreated Bednets

The earliest recorded use of bednets was in the 6th century BC in the Middle East. The motive for using the nets was, and for the most part still is, to allow the user to have a good night's sleep rather than to prevent disease (Lindsay and Gibson, 1988). As MacCormack (1984) points out, in some parts of west Africa physical barriers against night biting insects have been used for centuries.

The belief that bednets afforded protection from malaria rose more than 70 years before Manson suggested that malaria was transmitted by mosquitoes (Lindsay and Gibson, 1988) yet there have been very few studies of whether bednets actually

protect people from malaria.

From a retrospective study in The Gambia, Bradley *et al* (1986) reported that there was a significant inverse correlation between splenomegaly and use of untreated bednets even after allowances had been made for the effects of age, ethnic group and place of residence. They suggested that bednets (untreated or treated with permethrin) should be investigated further as a means of malaria control in The Gambia. However, in an intervention study Snow *et al.* (1988 [a]) found that providing untreated nets to all the population of a village did not give a significant protective effect against malaria in children. As a partial explanation of the disagreement of the retrospective and intervention studies, it was suggested that in the retrospective study there may have been diversion of mosquitoes to unprotected children since the controls (i.e. people with no bednet) were in the same village; this could have amplified the difference between people with and without nets in a way which would not have applied in the intervention study using whole villages for the treatment and for the controls.

In a comparison in a Kenyan boarding school of untreated mosquito nets, proguanil hydrochloride chemoprophylaxis or placebo, it was found that the nets and proguanil reduced attack rates of *Plasmodium falciparum* by 97.3% and 77.1% when compared to the placebo, there being 1, 8 and 35 cases respectively during a 12 week follow-up (Nevill *et al.*, 1988). The authors point out however that the mosquitoes may have been diverted to the children unprotected by a mosquito net. It is worth emphasizing that the study was carried out in the disciplined environment of a boarding school. The area is described as having seasonal transmission although becoming stabilized due to irrigation schemes. The authors suggest that using an insecticide on nets and curtains might strengthen the vector barrier.

There was no difference in the frequency of attacks of malaria between children who used bednets and those who did not in the Congo in an area of holoendemic malaria (Trape *et al.*, 1987). In Suriname, Rozendaal *et al.* (1989) did not

find a clear reduction in malaria where untreated hammock nets were used.

Impregnated nets give better individual protection than unimpregnated nets for a number of reasons. Firstly, and most importantly, nets become easily torn and are not always mended, which allows access of the mosquitoes into the net for feeding. This either does not occur, or is greatly reduced, if the nets are treated. Snow *et al* (1987 [a]) observed that a total of only 10 mosquitoes was found in treated nets whereas 265 were found in a similar number of torn untreated nets. Also in The Gambia, Lindsay *et al*. (1989 [a]) reported that no mosquitoes were found in treated nets during 255 nights of observation, whereas 81 were found in untreated nets. Untreated nets have long been considered by entomologists a convenient place for the collection of blood-fed female mosquitoes in the morning (e.g. Port and Boreham, 1982). Secondly, if a number of people share a bed and consequently a bednet, if it is available, then it is quite likely that the limbs of the people on the outside of the bed will come into contact with the netting. Hossain and Curtis (1989 [b]) showed that mosquitoes do not feed on an arm pressed against a treated bednet although they do feed through an untreated net. Lastly, bednets may not be tucked in properly, "doorways" may not be adequately closed or mosquitoes may enter bednets through mattresses made of maize stalks etc. It appears that the excito-repellent and deterrent effects of pyrethroid treated nets reduce the chances of this occurring.

1.4 The History of Treated Bednets

The earliest description of nets acting as more than just a physical barrier to mosquitoes can probably be attributed to Herodotus in the 5th century B.C. and it portrays fishermen living in Egypt : "Everyman of them has a net, with which he catches fish by day, then creeps under it and so sleeps. If he sleeps wrapped in a garment or cloth, the gnats bite through it, but through the net they do not even try to bite" (translation of Godley, 1981). Perhaps the fishy smell of the nets acted as a mosquito repellent.

Not surprisingly, the idea of insecticide or repellent treated nets is not new. The earliest available reports of deliberate treatment of nets are from the USSR in the 1930's using lysol and a plant product, and are described by Pavlovsky (1941) and Blagovchensky *et al.*, (1945). Then in the Second World War the American armies in the Pacific impregnated bed nets with 5% DDT in kerosene, applied by power driven paint sprayers or chemical warfare decontamination sprayers (Harper *et al.*, 1947). Six men were able to spray about 60-100 nets per hour with hand sprayers and twice this number with power driven sprayers. They state that bednets proved to be most valuable single measure against mosquito bites. Nauck *et al.*, (1948), described the use by the German army in Greece of Gesarol (1-5% DDT formulation) in water against the vectors of sandfly fever. Deposits of 0.8-8.0 g/m² were used for the impregnation of bednets. With Gix (DDT), it appeared that sandflies were affected by deposits without contacting them thus impregnated bednets were sufficient to control biting.

It seems strange then that the treatment of bednets was not reported again for almost 20 years but the reason was probably the preoccupation with DDT wall spraying. In 1967 Gouck *et al.* showed that 4 mesh per inch cotton netting treated with repellents gave a high degree of protection against the black salt marsh mosquito, Aedes taeniorhynchus. They also pointed out that the wide mesh netting permitted better air circulation than the smaller mesh sizes commonly used for bednets. In similar studies, Gouck and Moussa (1969), tested nets treated with 0.5g deet/g netting and a mixture of benzyl benzoate (30%), N-butylacetanilide (30%), 2 butyl-1,3-propanediol (30%) and emulsifier (10%). Both treatments provided complete protection against Culex quinquefasciatus and Ae. aegypti for 15 weeks. McDonald and Grothaus (1973) tested wide mesh nets (4 mesh per inch) with or without deet, and no net at all. They found no mosquitoes near the deet treated nets but people under untreated nets and with no net received bites, although the untreated net offered a small degree of protection.

In a test of three repellents on the same wide mesh netting the recommendation was 2-[p-methoxy-benzyl oxy] - N,N-dipropylacetamide for use in bednets, window

and door coverings etc. (Grothaus et al., 1974). The authors stressed that it would not be a good choice for the treatment of clothing because some skin irritation might result. They also pointed out that people were only just beginning to realise the potential value of space repellents and wide mesh netting as a tool for combating vector borne disease.

Brun and Sales (1976) were probably the first to evaluate insecticides other than DDT on bednets. Four organophosphates, fenitrothion, chlorpyrifos-methyl, chlorphoxim and pirimiphos-methyl, were tested on cotton nets in experimental huts. They concluded that although only chlorphoxim showed satisfactory residual properties (exceeding 85 % mortality for over two months) after an initial impregnation of 0.2 g/m², further evaluations should be carried out using net impregnated with the compounds found to be best for residual house spraying. They also pointed out that the large scale use of treated mosquito nets in a village or region could substantially reduce man/vector contact and thereby reduce the epidemiological risks of transmission. Also, they had already noticed in 1976 the point made above, i.e. that the concentrations used and the area treated are much lower than in the interior spraying of dwellings which might, they estimated, reduce insecticide costs by 80-90%.

The first workers to test synthetic pyrethroids on bednets were probably Hervy and Sales (1980). They evaluated permethrin and deltamethrin on three fabrics. Using one hour bioassays and Ae. aegypti they found that permethrin performed equally well on cotton as on synthetic netting. They reported that deltamethrin performed better on cotton than on synthetic netting. They stressed that only a field trial could determine the true values of the "efficient concentrations" and the "deterrent effect" which is especially important with the pyrethroid group of insecticides.

1.5 Personal protection using repellents

During the late 1970's and early 1980's a large number of trials were carried out on the impregnation of clothing. The two compounds most studied were diethyl-

m-toluamide (deet) and permethrin. A repellent is defined as " a chemical which causes insects to make orientated movements away from its source" (Dethier,

1956). Repellents may be divided into two classes depending on how they afford protection. Those materials that are sufficiently volatile to keep an insect at a distance are designated vapour or olfactory repellents (e.g.deet) and those that are so slightly volatile that insects must approach and touch the treated surfaces before being repelled are called contact or gustatory repellents. Permethrin is considered to be repellent by a number of workers although precisely which class it belongs to is not stipulated. It may also be called a deterrent (De Zulueta and Cullen, 1963) if it deters insects from entering a house where a treated net is used (section 1.14 and Chapter 6).

Permethrin was found to be an effective clothing treatment for protection against a broad range of blood-feeding arthropods such as human lice (Nassif and Kamel,1977), blackflies (Lindsay and McAndless, 1978), ticks (e.g. Schreck *et al.*, 1980 [a]) and mosquitoes (e.g. Schreck, 1984). Schreck *et al.*, (1977, 1978 [a], [b], 1980 [a], [b], 1982 [a], [b], [c] and 1984) have pioneered the work on the impregnation of clothing (especially military fatigues) with deet and permethrin which were applied in solutions of acetone or as pressurized sprays. They did not find that permethrin treated clothing gave the protection expected against sandflies, blaming sandfly behaviour and resistance to quick knock-down for the large number of bites recorded (1982 [a]). Maximum protection from bites would require application of deet to exposed skin when wearing permethrin treated clothing.

In field tests with deet-treated wide mesh cotton netting, Zaugg (1978) reported that collections from CDC miniature light-traps placed inside the netting showed that it was effective against mosquitoes, Culicoides spp. and sandflies for about 64 days.

Insect repellent jackets have been tested by a number of workers. Net jackets treated with 0.25g deet per g of netting provided protection against a variety of blood feeding Dipteran species for at least six weeks (Grothaus *et al.*, 1976). Lindsay and

McAndless (1978) treated wide mesh jackets with permethrin at 0.07g active ingredient (a.i.)/g of netting and found them to be less effective against blackflies and mosquitos than those treated with deet at 0.25 g a.i./g of netting. However, the toxic action of the pyrethroid treated jackets reduced the blackfly and mosquito population in a given area after approximately 10 minutes exposure, after which effective protection was afforded.

A one minute application by pressurised spray of 0.5% permethrin to clothing provided 100% protection against the Lone Star and American Dog ticks. A comparable application of deet (20%) provided 85% protection against Lone Star and 94% protection against American Dog ticks (Mount and Snoddy, 1983).

The repellency and toxicity of permethrin as a clothing impregnant against the Pacific Coast tick and the Pajaroello tick was tested by Lane and Anderson (1984). When offered a choice of permethrin treated or untreated surfaces to crawl on, most ticks gravitated towards the untreated surface within one or two minutes. However, a comparison of tick numbers found on treated and untreated overalls after the wearer had walked through infested grasslands showed that the numbers were not significantly different. Ticks removed from this clothing showed a highly significantly greater mortality one day later when the overalls had been impregnated.

Curtis *et al.*, (1987), found that deet impregnated anklecloths provided 83-94% protection against the important vector species An.gambiae s.l., An. funestus and Cx.quinquefasciatus. One impregnation remained effective for 72 days. They pointed out that most mosquito biting of individuals seated in chairs is on the ankles and feet, but that the wearing of impregnated anklets did not cause mosquitoes to bite higher up the body but drove most mosquitoes away altogether. They estimated that it would cost 26p a year to keep one pair of anklets impregnated with deet.

1.6 Pyrethroids for the Impregnation of Bednets

The high insecticidal activity, low mammalian toxicity and rapid environmental degradation of pyrethroids (Elliot *et al.*, 1978) made them the insecticides of choice for the impregnation of bednets.

Deltamethrin is 600 times as active, weight for weight, against *An.stephensi* as DDT and hundreds of times more toxic than dieldrin as a residual application against tsetse (Zerba, 1988).

Permethrin and deltamethrin have been most widely used (Curtis *et al.*, 1989) and are both classified by WHO as moderately hazardous (WHO 1986). To date there have been no complaints by bednet users of adverse side effects. A tingling (parathæsia) may be experienced on handling the emulsifiable concentrate (E.C.) or diluted emulsion of deltamethrin when dipping nets (if rubber gloves are not worn), but this soon wears off.

Because of their hydrophobic nature, pyrethroids are virtually immobile in soil allowing them to be readily degraded by aerobic conditions. Half-lives ranging from 1 day to 16 weeks are reported (Leahey, 1985).

1.7 Pyrethroids

A compound is considered to be a pyrethroid if its structure can be reasonably derived from that of the natural pyrethrins, and if it exhibits a range of biological properties that overlap to a considerable degree with those of existing members of the group (Davies, 1985). Natural pyrethrins come from chrysanthemum (*Chrysanthemum cinerariaefolium*) heads and have been used for centuries as insecticides.

The first synthetic pyrethroids in which characteristically high activity against insects and low mammalian toxicity were combined with greatly increased stability were described in 1973 (Elliot *et al.*, 1973).

The insecticidal activity of the pyrethroids depends upon the stereochemistry of their molecules. Pyrethroids are carboxylic acid esters (except for a group of

oxime esters, not yet exploited commercially). Because of their structures, several isomers are possible and not all the same isomers of a given compound display the same biological activity. Generally, the cis isomers of 3-phenoxybenzyl alcohols, and their alpha-cyano-substituted analogues are more active biologically than the corresponding trans isomers.

Pyrethroids, like DDT, have a negative temperature coefficient, i.e. their toxicity decreases with increasing temperature.

1.8 Mode of action of pyrethroids

Pyrethroids are neurotoxic to insects although their precise mode of action at the molecular level has not been fully elucidated (Miller and Adams, 1982). They act at the nerve membrane to modify the sodium channels - probably by impeding protein conformational changes at the lipid-protein interface (Zerba, 1988).

It was first reported by Narahashi (1977), that there appear to be two types of compounds - (i) those that caused excitation in the abdominal nerve cord of the crayfish with a clear cut relationship to their insecticidal properties (i.e. the more neuro-excitant they are, the more insecticidal they are), and (ii) those that show no overt excitation action, yet are still insecticidal. Thus the pyrethroids currently available on the world market can be classified into:

- a) those with good early knock-down but comparatively poor kill, e.g. permethrin.
- b) those with good kill but poor knock-down e.g. alpha-cyano pyrethroids such as deltamethrin and lambda-cyhalothrin.

An extensive survey of the effects of various pyrethroid analogues by using the cercal sensory nerves of cockroaches also concluded that there were two types of pyrethroids (Gammon *et al.*, 1981). Type 1 compounds, including permethrin, allethrin, and phenothrin do not have a cyano group at the alpha position and act mainly on the peripheral nervous system causing poisoning symptoms of restlessness, incoordination, hyperactivity, prostration and paralysis. Type 2

compounds, including deltamethrin, cypermethrin, fenvalerate and lambda-cyhalothrin have a cyano group at the alpha position (i.e. cyanophenoxyl benzyl pyrethroids), do not induce repetitive firing in the cercal sensory nerves and cause different symptoms. They act mainly on the central nervous system, producing hypersensitivity, tremors, paralysis and death.

In fact, Clements and May (1977) showed that the occurrence of repetitive firing induced by a large number of pyrethroids in the locust peripheral nervous system depends on both pyrethroid structure and the site of measurement.

Vijverberg *et al.* (1986) suggest that the modification of the sodium channels is the main mechanism of action of all pyrethroids and is responsible for their excitatory effects. Their results do not support the view that pyrethroids can be divided into two separate classes with distinct modes of action.

Since there are exceptions to the categories mentioned above, it is probably better to think of the compounds as showing a spectrum of activity.

The preceding studies on the impregnation of clothing and netting may have caused interest to be renewed in the concept of impregnating bednets for vector control and, coupled with the general disillusionment with other vector control methods led to the studies reviewed in the following pages.

1.9 Field Trials - Experimental hut and Entomological Studies

Darriet *et al.* (1984) carried out experimental hut trials in Burkina Faso in 1983. Cotton nets were impregnated with permethrin at a concentration of 0.08 g/m^2 ; this concentration was selected in the light of the studies of Hervy and Sales (1980). They reported (a) a reduction of about 70% in the number of Anopheles gambiae and An. funestus entering the huts, (b) an increase in the proportion exiting from 25-30% in control huts to 97% in huts with impregnated nets, (c) a reduction in proportion biting by 10 - 20% of that in the control huts, and (d) mortality of 20% due to the treated nets.

Several investigators have found reductions in the numbers of mosquitoes resting in houses with treated nets. In a coastal village in Papua New Guinea where bednets had been impregnated with permethrin at 0.4g/m^2 , the number of An.farauti collected resting and the human blood index of the engorged females were significantly reduced. Survival rates of An.koliensis were affected by the nets, but recruitment to the population was not. The peak time of biting was found to have shifted to early in the night which was probably because females returning from ovipositing were prevented from feeding by treated nets and therefore delayed their feed until early the following evening (Charlwood and Graves, 1987). Shifts in feeding patterns to earlier evening or dawn when many people would be unprotected by nets should be monitored closely since this would seriously reduce the effectiveness of treated nets.

A significant reduction in the numbers of An. punctulatus females resting in houses in which bednets treated at 0.5g/m^2 were used, compared to houses with untreated nets or no nets, was found by Charlwood and Dagaró (1987). Mark and release experiments indicated that this reduction was due to the deterrence of mosquitos from entering the houses because, when engorged females were released inside the houses, there were no differences in the proportions of females resting in houses with treated or untreated nets. Similarly Snow et al. (1987 [a]) found that numbers of An. gambiae in rooms containing permethrin treated nets were much lower than in rooms containing placebo treated nets. There was also a higher rate of exophily in the former case, yet the mortality and proportion fed were not significantly different.

A series of elegant studies in experimental huts were carried out by Lines et al (1987) to assess the efficacy of permethrin impregnated nets and curtains. Treated bednets killed more mosquitoes than untreated nets and increased the tendency of survivors to exit in the night. Treated cotton did not perform as well as treated nylon. Although this paper has more than once been quoted as not showing any appreciable

Table 1 Field trials carried out in China using impregnated nets.

Date	Region	No. of people	Insecticide/ Deposit density	Effects on malaria/ Reference in parentheses
1985 ^{1(a)}	Guangdong (Buji)	3000	D - W.P. 15mg/m ²	Reimpregnated 1 year later M.I. July-Dec 1984 reduced by 90% 1986
1986 ^{1(b)}	expanded to 32 000 (84% of people under treated nets)			M.I. April-Dec reduced by 65% (Li Zuzi, 1989)
1986	(Shenzhen)	160 000 (91% of people under treated nets)		M.I. April-Oct reduced by 65% compared with 1985 (Curtis <i>et al.</i> , 1989)
1986 ²	Jiangsu	22 700 (93% of people under treated nets)	D-E.C. 15mg/m ²	M.I. reduced by 87%
		26 000 (82% of people under treated nets)	P-E.C. 200mg/m ²	M.I. reduced by 86% (Curtis <i>et al.</i> , 1989)
1986 ³	Sichuan	30,000	D - W.P. 9.6 mg/m ²	M.I. significantly reduced (Curtis <i>et al.</i> , 1989)
1987	expanded to 2.48 million			
1986 ⁴	Hainan	4000	D - E.C. 25 mg/m ²	
1987			Repeated	Progressive decline in parasite rate over 2 years post net treatment and disappearance of <i>P. falciparum</i> (Curtis <i>et al.</i> 1989)
*M.I. = Malaria incidence		P = permethrin E.C. = Emulsifiable concentrate		D = deltamethrin W.P. = Wettable Powder

Notes on vectors

- 1(a) and 1(b) - Combined numbers of *Anopheles sinensis* and *An. anthropophagus* collected indoors on bednets reduced by 93% compared with pre-treatment data.
- 1 (a) and (b) - Outdoor densities of both species reduced by about 50%
- 2 - The total number of mosquitoes caught in the control, deltamethrin and permethrin treated villages after 11 collections carried out at intervals up to 104 days after treatment were 682, 29 and 40 respectively.
- 3 - Numbers of *An. anthropophagus* and *An. sinensis* found resting on and in the nets reduced by 99 and 94% respectively. Outdoor biting rates decreased by 98 and 91% respectively.

deterrence (i.e. reduction in the numbers of mosquitoes entering huts), in one set of experiments using nets or curtains treated with 0.2 or 1.0g/m², between 37 and 67% deterrence of *An.arabiensis* was found when compared with huts with no net. They also found that when an unprotected child slept near a child protected by a net, it received fewer bites than if neither had been under a net.

However, unlike Darriet *et al.* (1984) and Lines *et al.* (1987), Ree (1988) did not find any reduction in the numbers of mosquitoes entering a hut where nets treated at 0.5 or 0.2 g/m² were used, as compared with a control net. 88.2% and 84% mortality were obtained with the two treated nets respectively. Only two nights of observations were made for each treatment and there were no data on night-to-night variation in mosquito numbers.

In a trial in Orissa, India, using nets impregnated with permethrin at 0.5g/m² the per man hour density of *An. culicifacies* was reported to be significantly reduced in the intervention village (Jambulingam *et al.*, 1989). However, there is little data on pre-intervention density and the numbers in the control village dropped drastically at the end of the trial so these results are not very convincing.

A net (not in regular domestic use) which had been impregnated with 25 mg/m² deltamethrin caused 92% mortality, assessed using 1 hour bioassay, 2 years after impregnation. Between 18 and 41 days after impregnation of a net with 2g/m² DDT there was only 54.8% mortality (Li Zuzi *et al.*, 1987).

1.10 Village Scale Field Trials

There was probably research on bednets in China in the early 1980's, but details of their field trials did not become available until the mid 1980's. Even the most sceptical commentators (e.g. Molineaux in WHO., 1989) on the effects of impregnated materials on malaria in humans agree that the Chinese experience appears to be a success (see Table 1). Nevertheless, it was stressed (WHO, 1989) that China has the advantages of an integrated and very active control programme

(spraying DDT was only comparatively recently beginning to fail in some regions) and a low level of endemicity in comparison with tropical Africa.

Deltamethrin impregnated nets have been put into operational use in Guangdong and Sichuan provinces in China (Table 1 and Figure 1). In Guangdong, impregnated nets were considered by the people to be far more acceptable than DDT spraying. The deltamethrin was applied using sprayers when the control programme was extended to 34 counties in Sichuan in 1988.

In a trial carried out in The Gambia, a sample of the children in a village who slept under treated nets (0.5g permethrin/m²) had significantly fewer episodes of clinical malaria than children sleeping under placebo treated nets. However, at the end of the malaria transmission season, there was no significant difference in the prevalence of splenomegaly or parasitaemia between the groups (Snow *et al.*, 1987 [b]). The authors suggest that permethrin treated nets may have a greater effect on the success of feeding than on the number of bites by infected mosquitoes i.e. mosquitoes may become irritated by the insecticide and leave before taking a full meal. This may cause a reduction in parasite inoculum due to treated nets. In a subsequent trial in which all the nets in the village were impregnated, the authors reported a reduction in febrile episodes with parasitaemia of about 63% (Snow *et al.* 1988 [b]).

The results of a trial in Papua New Guinea showed that the incidence of Plasmodium falciparum between surveys four weeks and 10 weeks after clearance of parasitaemia with Fansidar was significantly lower among the 0 to 4 year olds in villages with impregnated nets (0.5 g permethrin/m²) than with unimpregnated nets. Impregnated nets had no effect on the incidence of P. falciparum in 5 to 9 year olds or on that of P. vivax among the 0 to 4 or 5 to 9 year olds. Sporozoite rates in females of the An. punctulatus complex decreased significantly after impregnation in two out of the four experimental villages (Graves *et al.*, 1987).

In a small scale trial carried out in Sabah, Malaysia, nylon nets with cotton borders were impregnated with about 0.062g permethrin/m². Fansidar was used to clear parasitaemia. The parasite rates in children recovered more slowly in the

villages with the impregnated nets than in the control village without nets but after only 2-4 months the rates in the treated and control villages were similar. Mosquito collections inside treated bednets were significantly reduced for at least 217 days but the project failed to achieve prolonged suppression of malaria transmission for a combination of entomological, sociological and practical reasons (Hii *et al.*, 1987).

Carnevale *et al.* (1988) reported on a trial carried out in Burkina Faso. They estimated that there was a 91% reduction in sporozoite inoculation rates by the *Anopheles gambiae* complex and *An. funestus* and that the risk of a malaria attack was reduced by 59% as a result of using an impregnated net (25mg/m² deltamethrin), which is similar to the reduction calculated by Snow *et al.*, 1988 [b]).

1.11 Why people like treated nets

In a trial in The Gambia 97% (103/106) of mothers from villages where permethrin treated nets had been used liked them very much, whereas only 10 % (12/121) of people where the nets had been dipped in placebo gave this response (Snow *et al.*, 1987 [b]). Reasons given for liking treated nets included reductions in headaches and sweating (Snow *et al.*, 1988 [b]).

Impregnated bednets (0.4g permethrin/m²) provided effective control of bedbugs for 10 weeks in Papua New Guinea. The breakdown of this control was blamed on loss of treated nets and influx of unprotected people (Charlwood and Dagar, 1989). The authors state that control of headlice and bedbugs was the main reason why people wanted their bednets re-treated and would carry them several miles to have this done.

The difference in the prevalence of bedbugs before and after impregnating nets with permethrin was found to be highly significant in a study carried out in The Gambia. Ticks were eradicated from households with permethrin treated nets but not placebo treated nets. There was a reduction of 78.6 % of headlice in the intervention group after treatment, a reduction of 86.4 % of flying insects and 42.2 % of crawling

insects (Lindsay *et al.*, 1989 [b]). The authors point out that these effects on domiciliary arthropods should encourage the acceptance of pyrethroid by a community. It would be interesting to carry out a follow up study a year or so later.

1.12 Field Trials of materials other than bednets

In some situations, for example, where bednets are too expensive for, or not acceptable to the population, impregnated curtains or wide mesh netting may be a suitable alternative. Majori *et al.* (1987) found that cotton wide-mesh netting curtains impregnated with 1.0 g permethrin/m², around the eaves and over the doors and windows, caused reductions in indoor resting density of *An. gambiae* s.l of 98.6% and 98.8% after 1 and 3 months respectively. After 11 months, the reduction in numbers of *An.gambiae* s.l. was 54.4%, the lower efficacy being attributed to permethrin decay and the curtains becoming overlain with dirt and cobwebs.

A study using wide mesh netting (40x40mm and 10x10mm) impregnated with phenothrin at 0.5g/m² was carried out by Kurihara *et al.* (1986). The experiment was carried out in a pigsty. In two sections of the sty insecticide treated nets, 3 weeks post impregnation, were used and in the other two sections small and large mesh untreated nets were used. Light traps within either enclosures were used to catch the mosquitoes which entered. The treated netting was found to reduce the proportion of surviving mosquitos to a negligible level and also greatly reduce the proportion of fed mosquitos. Only the 10x10 mm netting showed a strong deterrent effect, with less than half the number of mosquitos found when the untreated nets were used. Although only two nights data are presented (and so the positions of the treatments could not be compared) the results appear decisive.

A study, in which cotton curtains with a 0.5 cm mesh impregnated with 1.0g permethrin/m² were used, found a 99 % reduction in the overall indoor density of sandflies. There was no equivalent reduction in fly numbers in houses fitted with untreated curtains (Majori *et al.*, 1989). The authors suggested that this method could

be an effective alternative to residual house spraying for the control of endophilic vectors of leishmaniasis. However they recommended a pyrethroid impregnated screen where personal protection against leishmaniasis is a priority since sandflies can pass through a 0.5 cm mesh and might bite before dying. Unfortunately no data on other vectors was presented.

1.13 Laboratory Studies on Treated Netting

Loong *et al.* (1985) tested permethrin and DDT impregnated nets. The nylon nets were sprayed using a Hudson sprayer as the authors believed the dipping method would give irregular coverage with this fibre. Unfortunately, no data are given for the coverage obtained by spraying. Laboratory evaluation of the treated netting was carried out by releasing An. maculatus into the treated nets for 30 minutes or exposing mosquitos for 10 minutes to the outside of the nets. Both methods gave almost 100% mortality up to one year post-treatment. Even after one wash, the nets continued to give very good results.

In an evaluation of seven insecticides for insecticidal activity against Culex pipiens, after nine months of ageing of the treated netting, cyphenothrin was the most effective, causing 100% mortality. It must be stressed that the exposure time was 24 hours which is an excessively long time to test treated netting (Itoh *et al.*, 1986). When the mesh size was less than the wing span, the mosquitos were seen to rest on the netting before they passed through, causing them to pick up a lethal dose of insecticide.

A 12 hour exposure to test the fumigant effect (i.e. kill without contact, also called vapour toxicity - section 4.4) against An. farauti gave 100 % mortality with 0.5g permethrin/m² and 93.3% mortality with 0.2g/m². Mortality rates of An. maculatus after 1.5, 3, 6 and 12 hours exposure to 0.5g/m² were 89.8, 90, 94.9, and 100 % respectively. All the tests were carried out within ten days of treatment of the nets and the cage containing the mosquitoes was held 30-50 cm from the treated

netting (Rec, 1986). Such a strong vapour toxicity has not been found by other workers to date.

The behavioural effects of impregnated netting on insects have been little studied in comparison with their insecticidal effects. Kurihara (1988) discussed a method of assessing behavioural reactions to insecticide treated netting. Phenothrin treated netting had a strong deterrent effect and inhibited feeding of An. stephensi. However Cx. pipiens was not deterred from entering. Feeding in Ae. albopictus was less inhibited than in 3 other mosquito species.

Hossain and Curtis (1989 [b]) tested the effect of a human subject sitting under a nylon or cotton net (1.5mm mesh) and pressing his arm against the net. When the net was impregnated with 0.2 g permethrin/m² mosquitos did not obtain a blood meal and they all subsequently died. Wide mesh netting (4 and 8mm) had similar effects. However, with an untreated net, 64.5% of mosquitos fed and none died.

1.14 Washing

Washing impregnated nets generally has a detrimental effect on retention of the insecticide by the net and on mosquito mortality. Four warm water detergent washes in a machine reduced the amount of permethrin retained by 49 % (Schreck et al. 1982 (c)).

Handwashing by Gambian women severely reduced the toxicity as shown with three minute bioassays. For example, on synthetic netting, impregnated at a target concentration of 0.5g/m², mortality was 85.9% before washing and 24.7% after washing. The permethrin content was approximately halved as shown by gas liquid chromatography (Snow et al., 1987 [a]).

Burgess et al. (1988) reported chemical analyses which showed that hand washes in hot water removed permethrin efficiently from dip-treated fabrics. More than 50% of applied permethrin was removed by three washes and all of it was removed by 10 washes. Machine washes removed only 30% of permethrin after three

washes.

In a survey carried out in Orissa, India, after five months, 57% of impregnated nets had been washed three or more times (Jambulingam *et al.*, 1989).

Lindsay *et al.*, (in press), compared 12 pyrethroid preparations and found that washing greatly reduced the concentrations of all the compounds on the netting. Cypermethrin, lambda cyhalothrin and permethrin applied in a hot acid solution had the highest insecticidal activity after washing.

The Ndjuka in Suriname wash their cotton sheeting hammock "nets" once a week. One wash caused a 70% reduction in insecticidal activity as determined by knock-down (Rozendaal *et al.*, 1989).

1.15 Control of other diseases

Impregnated bednets have been aimed almost exclusively at malaria vector control although there have been a few studies on sandflies (see section 1.12). However, the theory may be applicable to other vector species, especially the vectors of filariasis where transmission occurs at night as a consequence of the nocturnal periodicity of most Wuchereria bancrofti and Brugia malayi infections.

The much lower efficiency of transmission of filariasis improves prospects for disease control by vector control, provided that the vector control can be maintained for years.

Interactions between humans and mosquitoes usually decrease as socioeconomic conditions improve due to the elimination of breeding sites and an increase in barriers to prevent contact between humans and mosquitos e.g., screens (Grove, 1986).

Bancroftian filariasis was once endemic in northeast Australia and southeast USA but disappeared spontaneously with improved socioeconomic circumstances. However this is unlikely to occur for many years in developing countries where filariasis is endemic. In fact, filariasis is thought to be on the increase because of

increasing urbanization and associated water borne sewage providing breeding sites for Cx. quinquefasciatus (Curtis and Feachem, 1981). Mass use of mosquito nets may have considerable potential for the reduction in the prevalence and intensity of microfilaremia and perhaps even for bringing about the ultimate eradication of filariasis (Grove, 1986).

The only other paper published to date purporting to be on the effects of impregnated materials on a disease other than malaria is Charlwood and Dagaro's "Impregnated Bednets for the control of Filariasis transmitted by Anopheles punctulatus in rural Papua New Guinea" (1986). Unfortunately there are no data given on control of disease transmission, only the expression of the opinion that the results on numbers of mosquitoes caught in houses with and without treated nets indicate that impregnated nets may have the potential for control, since filariasis is an inefficiently transmitted disease.

1.12 Resistance to pyrethroids

The future of pyrethroid impregnated bednets will be influenced both by their ability to control vectors resistant to currently used insecticides and by the extent to which pest populations develop economically significant levels of resistance to pyrethroids. It is probably true to say that very little thought is given to the possibility of resistance until it is too late, probably because it is not known how to manage resistance.

The widespread use of impregnated nets could quite feasibly select for resistant populations of mosquitoes. The bednet situation has been likened to the use of pyrethroid impregnated ear tags on cattle. Initially excellent control of the horn fly, Haematobia irritans, was achieved but after just two to three years resistance was widespread throughout the USA (Byford and Sparks, 1987). Only females are attracted to treated netting whereas both sexes of horn flies are attracted to cattle. The threat of pyrethroid resistance due to the widespread use of impregnated nets should not be

ignored inspite of the conclusion by Malcolm in 1988 that physiological resistance of anophelines is less widespread than previous commentaries suggest (eg Georghiou, 1986; Brown 1986).

However, recently, Curtis *et al.* (in press) found that although a permethrin resistant strain of *Anopheles stephensi* had a limited ability to penetrate a permethrin net this was less so with a deltamethrin net. Mortality of the mosquitoes was higher after contact with the deltamethrin impregnated net: approximately 75% compared with only about 15% with the permethrin net. When this resistance was heterozygous, it gave no protection against permethrin impregnated netting and, perhaps for this reason, there was no appreciable response after 7 generations of selection for females which succeeded in penetrating a permethrin impregnated net. Thus they concluded that the threat of this form of resistance may not be as great as was once feared.

As Curtis (1987) points out, methods proposed for managing resistance appear to have more chance of success if introduced when resistance genes are rare. The methods frequently proposed for controlling/ delaying the evolution of resistance are i) Replacement of failed insecticides by new ones (ii) Rotations of different insecticides and (iii) mixtures of different insecticides. Theoretical studies suggest that mixtures should be more effective than rotations in preventing resistance (Curtis, 1985).

1.13 Summary of the Introduction

There are many reasons for the growing interest over the last five years in the use of insecticide impregnated nets to control vector-borne diseases, especially malaria. These include (i) the effectiveness of impregnated nets at killing or deterring mosquitoes from entering houses, (ii) simplicity of treatment and relative cheapness compared to residual house-spraying and (iii) beneficial side effects such as mortality of head lice and bed bugs.

Although research into nets treated with DDT, anti-septic or repellents has

been sporadically carried out since the 1930's, the trials leading to the widespread use summarized in Figure 1, did not occur until the early 1980's. This was due to the availability of residual pyrethroids coinciding with increasing problems of resistance to DDT and chloroquine. Bednet use is very variable in different parts of the world. Nets have been used in some countries for centuries prevent mosquitoes biting people and being a nuisance at night. Untreated nets are often found to contain blood-fed females in the morning, but mosquitoes are scarcely ever found in treated nets.

The pyrethroid characteristics of high insecticidal activity, low mammalian toxicity and rapid environmental degradation made them a suitable choice for the impregnation of bednets.

Experimental hut trials reported entomological findings such as reduced entry (deterency) into huts containing treated nets, increased mortality, reduced feeding and increased exiting of mosquitoes compared to huts containing untreated nets.

Village and operational trials have assessed both entomological and parasitological effects. Important findings are reductions in clinical attack of malaria.

A serious drawback to programmes of treating bednets is that if they are washed, much of the insecticide is lost, with a resulting decrease in toxicity to insects.

1.14 Objectives of this study

Despite the considerable number of field trials on permethrin and deltamethrin impregnated nets, there has been very little laboratory work on the relative efficacies of the different pyrethroids or fibres. Thus the studies in Chapter 2 were undertaken to establish the optimum pyrethroid/fabric combination. The two most widely used pyrethroids, permethrin and deltamethrin, as well as lambda-cyhalothrin (an alpha cyano-pyrethroid similar to deltamethrin) are compared on the three fibres most available for bednet use, cotton, nylon and polyester. Because, in some countries, bednets are too expensive, or they are disliked because they restrict air circulation, the performance of treated polypropylene as tightly woven material suitable for wall

hangings or strands suitable for "curtains" was also assessed.

The Emulsifiable Concentrate (E.C.) formulation has been used almost exclusively in trials although a number of other formulations are available. In Chapter 3, a range of formulations were tested and compared with the performance of emulsions made from the ordinary E.C. As already discussed, the loss of active ingredient due to washing of nets is a serious problem where nets are washed regularly. Thus a wash-resistant formulation was made and compared with the other formulations in the laboratory. The effects of soap on pyrethroid loss and subsequent uptake of pyrethroid were examined and related to field practice. The effect was investigated of emulsion acidity, emulsion temperature and the time spent by netting in the emulsion on fibre pyrethroid uptake and retention of active ingredient after washing.

Chapter 4 investigated how side-effects of treated nets, such as knock-down, leg fracture, a vapour effect and feeding inhibition affect mosquitoes. The irritant effect on mosquitoes caused by treated cotton and nylon netting was compared. The time taken to pick up a lethal dose was determined and in Chapter 5, using video recording techniques, this was related to the time that mosquitoes spend on treated nets when searching for a blood meal. Deposit densities simulating decayed field deposits were studied in an attempt to relate this to the effective life of unwashed impregnated nets in the field.

Tests in experimental huts had previously been limited to permethrin and deltamethrin. In the present studies the range was extended to a comparison of nets treated with the pyrethroids, cypermethrin, deltamethrin, lambdacyhalothrin and permethrin and the organophosphate pirimiphos-methyl. The results are reported in Chapter 6. The main aim of the hut trial was to test the performance of washed and unwashed nets. The organophosphate was tested to compare its performance with the pyrethroids, with a view to using a mixture of 2 such unrelated compounds to forestall the build up of pyrethroid resistance. The wash-resistant formulations and a mixture

of permethrin and pirimiphos-methyl were also tested in experimental huts.

The final objective of the study was to compare the toxicity to mosquitoes of ordinary permethrin, wash-resistant permethrin and lambdacyhalothrin treated nets under normal conditions in a Gambian village. The treatments were compared using bednet searches and bioassays. The acceptability and popularity of treated nets was also investigated and the results are reported in Chapter 7.

In Chapter 8, conclusions from this study and suggestions for future work are made.

CHAPTER 2

STUDIES ON FABRIC/PYRETHROID COMBINATIONS

2.1 Relative efficacy of three pyrethroid insecticides for the treatment of mosquito bednets made from different fabrics

2.1.1 INTRODUCTION

There have been few investigations of the comparative suitability of the different fibres commonly used for making bednets: cotton, nylon, and polyester.

The structures of these fibres (shown in Appendix 2.1) are reviewed below.

2.1.1.1 Cotton

Cotton is the most important textile fibre in the world, despite inroads made into its markets by synthetic fibres. China, The Soviet Union and the United States are presently the three largest producers of cotton, accounting for two-thirds of the world crop (Peters, 1985).

The cotton fibre is a single cell, appearing under the microscope as an irregularly twisted, collapsed, flattened tube with a central canal (lumen) throughout its length. Purification of raw fibre by any of several chemical processes results in a material that is at least 99% cellulose. Elementary analysis of cellulose classifies it as a carbohydrate of empirical formula $C_6H_{10}O_5$. The cellulose molecule consists of a series of glucose rings joined together.

Water molecules can be attracted to the hydroxyl groups by hydrogen bonds. X-ray diffraction photographs of cellulose show both a regular pattern, characteristic of a crystalline material, and a diffuse halo, characteristic of a non-crystalline material. This division into two regions is confirmed by chemical evidence that only part of the fibre material is accessible to certain chemical reactions (Morton & Hearle, 1975).

The formation of the fibre occurs in the wet state, and it is thus greatly swollen. When drying occurs, the fibrils will associate, but like any crystallisation of

long chain units, appreciable disordering may occur owing to entanglement or mismatching of fibrils. On re-exposure to water (e.g. impregnation with pyrethroid emulsion) there is limited swelling but not solution of the material. On drying, the different regions of the cotton fibre collapse. Thus cotton contains internal void spaces. The density of fibres varies when they absorb water, e.g. with cotton the density decreases (1.55 g/m^2 to 1.52 g/m^2 at 65% r.h.) i.e. the fibres swell. The last two points are important when considering pyrethroid/cotton interaction (see later).

2.1.1.2 Nylon

Polyamides are macromolecules whose structural units are interlinked by the amide linkage -NHCO- . Synthetic polyamides with structural units derived predominantly from aliphatic monomers are members of the generic class of nylons (Peters, 1963).

They are partly crystalline materials i.e. they are characterised by both an unordered amorphous state and an ordered crystalline state. The behaviour of polyamide fibres is best explained by models that assume a regular alternation of crystalline and amorphous layers within a microfibrillar structure. Such a structure is the result of the extensional flow and plastic deformation to which polyamides are subjected in melt-spinning and drawing operations. The rate and extent of dyeing (or impregnation with pyrethroid) depends significantly on the morphology, crystallinity and molecular orientation of the fibre, as well as on constitutional parameters such as concentrations and reactivities of the amide groups and end groups (Peters, 1975).

2.1.1.3 Polyester

Polyester fibres are very similar to polyamide fibres, both being made in the same way, by melt-spinning and drawing, from linear condensation polymers. The definition of a polyester is: "a fibre composed of linear macromolecules containing at least 85% (by mass) in the chain of an ester of a diol and terephthalic acid".

The differences from polyamides are due more to the benzene ring. This gives stiffness to the chain and in particular, impedes deformation of disordered regions. Furthermore, although there may be an appreciable electronic interaction between neighbouring benzene rings, this is not as strong as the hydrogen-bonding in the polyamides (Morton & Hearle, 1975). Commercial polyethylene terephthalate (P.T.) fibres are normally semi-crystalline and may be represented as consisting of a spectrum of regions ranging from highly crystalline to virtually non-crystalline - depending on the processing history of the fibre. P.T. is by far the most important fibre in light-weight curtain nets, where its strength, resistance to photo-degradation behind glass and good colour retention are major advantages.

A large proportion of P.T. is marketed under the generic name "polyester". Some major producers of P.T. fibres and their trade names are; du Pont - "Dacron"; Hoechst - "Trevira" and ICI - "Terylene". The advantages of P.T. over other man-made fibres are such that it seems likely to consolidate its position as leader in this field. However, despite its advantages, it is usually more expensive than nylon and cotton for bed net use (Lewin & Pearce, 1985).

Fabrics used for field trials were either those already being used by the local population (e.g. Snow *et al.*, 1987 [b]) or those obtained specifically for the purpose of trials (e.g. Graves *et al.*, 1987).

Previous studies on the efficacy of different fibres as carriers of pyrethroids have given conflicting results. Hervy & Sales (1980) found cotton to be a more effective substrate than synthetic fibres, but they used an excessive exposure period of one hour for their bioassays. This would cause knockdown of some insects during exposure and continue to give 100% kill, even when the residue was much decayed. Hossain *et al.*, (1986), using the more realistic exposure of 2 minutes, found nylon to be better than cotton and as good as polyester when impregnated with permethrin. Also working with permethrin, Lines *et al.* (1987) found nylon netting killed better

than cotton. Snow *et al.* (1987 [a]) found differences between the toxicity to mosquitoes of 4 fabric types impregnated at the same deposit density of permethrin using a 3 minute exposure.

The following studies were therefore undertaken in order to:

- (i) further investigate pyrethroid/fabric combinations using bioassay mortality to assess effectiveness;
- (ii) to compare fabric types and pyrethroid deposits using scanning electron microscopy.

2.1.2 METHODS AND MATERIALS

2.1.2.1 Fabrics

Cotton - leno weave, open mesh, approximately 1 mm mesh size, weight 39.7 g/m² (Ahmedabad Cotton Co., India).

Nylon (polyamide) - bobbinet weave, approximately 1 mm mesh size, weight 23.7 g/m² (Small & Tidmas, Nottingham, U.K.).

Terylene (polyester) - bobbinet weave, approximately 1 mm mesh size, weight 20.8 g/m² (Small and Tidmas, Nottingham, U.K.).

2.1.2.2 Insecticides

The pyrethroids used were:

permethrin (25% w/v a.i. Emulsifiable Concentrate (E.C.), "Imperator", ICI Agrochemicals);

deltamethrin (2.5% w/v a.i. E.C., "Decis", Roussel Uclaf);

lambdacyhalothrin (2.5% w/v a.i. E.C., "Icon", ICI).

The structures are shown in Appendix 2.1

Deposit densities initially chosen for bioassay were based on the toxicity ratios found by Hervé (1985) in agricultural usage of pyrethroids. However, these were

modified to those found necessary to span the range from zero to 100% mortality with each compound.

The deposit densities on the netting were analysed using Gas Chromatography (G.C). The method of analysis used and examples of target and observed deposit densities are shown in Appendices 2.3 and 2.4 respectively.

2.1.2.3 Mosquitoes

Anopheles gambiae (strain G3 from The Gambia) was used for bioassay tests (see Appendix 2.2 for details of rearing). This strain is susceptible to all insecticides.

2.1.2.4 Impregnation of fabrics

Pieces of netting 30 x 36 cm were treated by dipping them in dilute pyrethroid emulsion and then wringing out excess liquid. The volume of water absorbed by each fabric was calculated from the difference in dry and wet weights. Assuming that the fibre shows no special affinity for pyrethroid, as Hossain and Curtis (1989 [a]) found for permethrin, then the amount of E.C. required to treat a given area of netting is:

$$\frac{\text{Target deposit density} \times \text{area of fabric} \times 100}{\% \text{ a.i in the E.C.}}$$

This amount of pyrethroid E.C. was made up with distilled water to the volume of emulsion necessary to wet the fabric. To ensure the netting was adequately and evenly saturated and to make the quantities of E.C. and water easier to handle, ten times the minimum required amount of diluted E.C. was prepared. Micropipettes were used to measure the very small volumes of deltamethrin and lambda-cyhalothrin E.C. After treatment, pieces of impregnated netting were laid flat on polythene sheeting indoors and left to dry for 24 hours. As the controls, pieces of fabric were dipped in distilled water, dried and used for bioassays.

2.1.2.5 Contact Bioassays

Rectangles of pyrethroid-treated and control netting measuring 12 x 15 cm were cut, stuck using autoclave tape to filter paper of the same size for support, and then inserted into the plastic exposure tube of World Health Organisation adult mosquito susceptibility test kits (WHO, 1970). The filter paper was used to line the tube to eliminate the possibility of absorption of pyrethroid into the plastic walls and also to provide a support for the more flimsy fibres, which would otherwise sag and introduce a variable exposure area. A plain piece of filter paper was placed in the recovery section of the kits. The kits were placed upright.

Groups of 15-20 glucose-fed adult female mosquitoes, 3-5 days old, were exposed for 3 minutes to a range of deposit densities of each pyrethroid on each of the three fibre types. A pad of cotton wool soaked in 10% glucose solution was placed on the gauze of the recovery tube to provide sustenance for the test mosquitoes during the recovery period. Knock-down was scored after 1 hour and mortality was scored after 24 hours. All tests were carried out in an insectary maintained at $24^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and $64\% \pm 2\%$ R.H.

Several replicates were carried out over a number of days. Replicates were carried out until a good fit to a linear regression of probit mortality on log dose was obtained. Mortality data were analysed using the probit/log dose regression program used in the London School of Hygiene and Tropical Medicine.

2.1.2.6 Electron Microscopy

A scanning electron microscope was used in an attempt to visualize pyrethroid deposits on the two most commonly used fibres, cotton and nylon. Approximately 20 mm x 20 mm sized pieces of netting were examined : (i) nylon treated with 0.8 g permethrin/m²; (ii) cotton treated with 0.8 g permethrin/m²; (iii) nylon treated with the same concentration of the blank E.C. (i.e. that containing all constituents of the

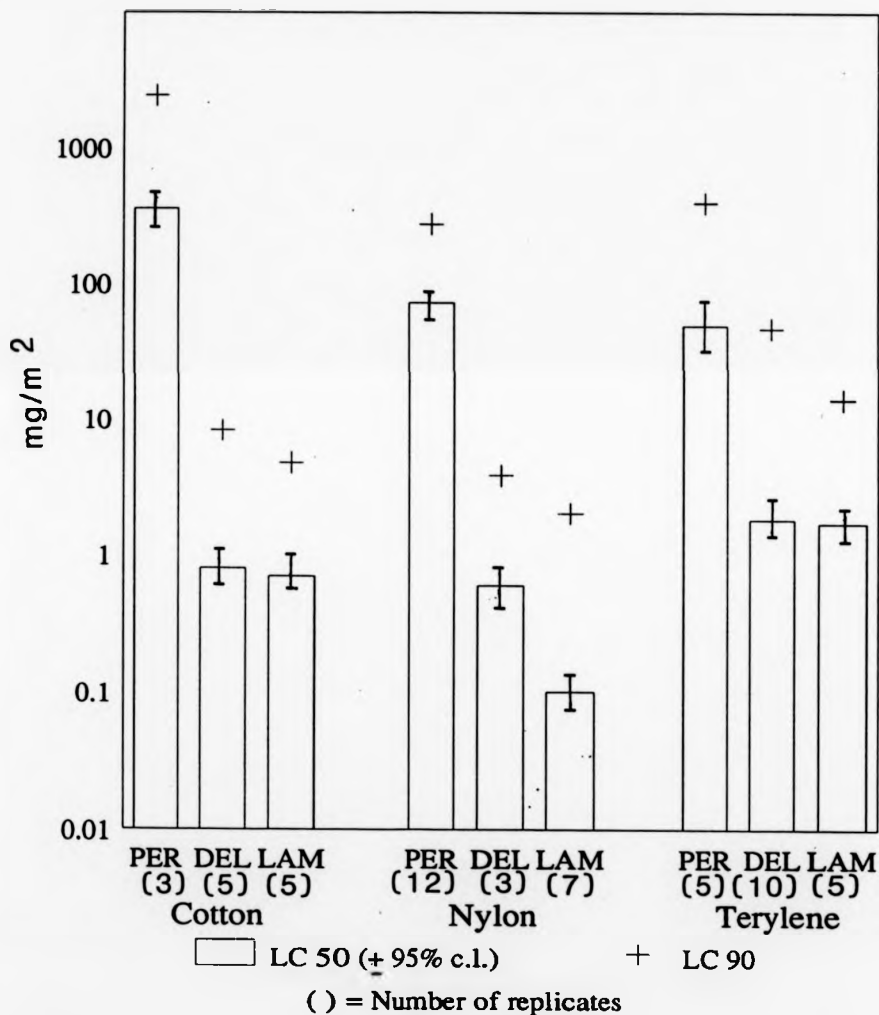
Table 2.1 (a)

Results of Bioassays on *Anopheles gambiae* using permethrin, lambdacyhalothrin and deltamethrin emulsifiable concentrates on cotton, nylon and Terylene netting.

PYRETHROID		FIBRE	
	COTTON	NYLON	TERYLENE
PERMETHRIN (25% E.C.)			
LC ₉₀	2410	270	390
LC ₅₀	350	72	49
95% C.L.	(270 - 450)	(64 - 80)	(35-64)
χ^2 (d.f.)	9.2 (6)	4.44 (5)	9.42 (5)
P	>5%	>10%	>5%
no. replicates	3	12	5
LAMBDA CYHALOTHRIN (2.5% E.C.)			
LC ₉₀	4.7	2.0	14
LC ₅₀	0.7	0.11	1.7
95% C.L.	(0.5 - 0.9)	(0.075 - 0.15)	(1.3 - 2.1)
χ^2 (d.f.)	4.2 (4)	3.43 (6)	8.07 (6)
P	>10%	>10%	>10%
no. replicates	5	7	5
DELTAMETHRIN (2.5% E.C.)			
LC ₉₀	8.2	3.8	47
LC ₅₀	0.89	0.6	1.8
95% C.L.	(0.61 - 1.3)	(0.4 - 0.8)	(1.4 - 2.1)
χ^2 (d.f.)	3.6 (4)	8.69 (4)	6.09 (6)
P	>10%	>5%	>10%
no. replicates	3	3	10

Note: all dosages in mg/m²

Figure 2.1 LC 50 ($\pm 95\%$ c.l.) and LC 90 values of permethrin, deltamethrin and lambdacyhalothrin on cotton, nylon and Terylene netting



formulation except the permethrin); (iv), (v) and (vi) nylon, cotton and Terylene dipped in water, and (vii) polypropylene (see 2.2), dipped in water. Each piece of netting material was temporarily mounted in a Balzers gold sputter coating unit and coated with approximately 20 nm of gold on both sides of the fabric. The procedure of coating both sides of the fabric was found necessary to prevent surface charging during observation in the Jeol JSM -35 scanning electron microscope.

Scanning Electron Micrographs were recorded onto Ilford FP4 film at an accelerating potential of 15 kV using a 100 s scan speed.

2.1.3 RESULTS

2.1.3.1 Bioassays of treated netting

The $LC_{50} \pm 95\%$ C.L. and LC_{90} with values of heterogeneity χ^2 , (with its significance level) and number of replicates are given in Table 2.1. The data are presented graphically in Figure 2.1. The LC_{50} and LC_{90} values for a given pyrethroid were higher on cotton than on nylon, although the confidence limits on deltamethrin on nylon overlapped with those on cotton. The LC_{50} for permethrin was about 5 times higher on cotton than nylon and the LC_{90} was about 9 times higher on cotton than nylon. The LC_{50} and LC_{90} values for lambda-cyhalothrin were 6 and 2 times as high on cotton as on nylon. The corresponding factors for deltamethrin were 1.5 and 2.2. There were huge differences in the efficacies of the alpha-cyano pyrethroids (deltamethrin and lambda-cyhalothrin) in comparison with permethrin, e.g. on nylon lambda-cyhalothrin was over 600 times more insecticidal than permethrin.

2.1.3.2 Electron Microscopy

See plates 1 and 2.

The micrographs in plate 2 show that a liquid film was visible around the permethrin-treated nylon fibres. This was not visible on cotton treated with

permethrin, on nylon treated with the blank E.C. formulation, or on nylon dipped in water. It is therefore concluded that the film consists of the active ingredient, permethrin. Plate 1 shows the untreated cotton fibres which appear very similar to the treated ones in plate 2, i.e., the permethrin is not visible on these rough fibres. Finishing materials (e.g. resins, polyvinyl acetate and starch) which are used to improve the appearance/durability of fibres are visible on the nylon, cotton and Terylene.

2.1.4 DISCUSSION

The following factors affect the uptake by an insect of an insecticide from a surface deposit: the dose (referred to as deposit density in this thesis), the formulation, the surface, the anatomy and behaviour of the insect and particularly, how long it spends on the treated surface (Chadwick, 1985). The present study used emulsifiable concentrates, 3-5 day old mosquitoes and 3 minute exposure periods. It should be pointed out that these relatively short exposure times (when compared to the standard WHO exposure period of one hour for susceptibility tests, WHO, 1970) were used for two reasons. Firstly it is desirable to avoid the insects being knocked-down during the exposure period, because this leads either to them falling off the deposit or to them lying on it. In either case the pickup by knocked down individuals is likely to be different from those not knocked down. Secondly, unconfined mosquitoes tend not to spend long periods in contact with pyrethroid treated fibres because of their excito-repellency; thus a one hour exposure in a bioassay is unrealistic (Hossain and Curtis, 1989 [b]; Chapter 5).

The type of surface provided by the different fibres for the pyrethroids is very important. As reported in the results, the fibres showed differences in bioassay performance. Possible reasons for this, relating to the structure and properties of the fibres, are now discussed.

2.1.4.1 Moisture Regain (M.R.) of fibres

All the natural animal and vegetable fibres have groups in their molecules which attract water, e.g. in the cellulose molecule each glucose residue contains 3 hydroxyl groups. However, all the synthetic fibres so far produced contain few, if any, hydrophilic groups and this accounts for their low moisture absorption (Schick, 1977).

Moisture regain (weight of moisture present in a textile material expressed as a percentage of its oven dry weight, [Morton and Hearle, 1975]) is an indicator of the absorptive capacity of fibres. If M.R. is ranked in decreasing order a clear pattern can be seen (Table 2.1(b).)

Cotton has the highest M.R. and is followed by nylon, and then Terylene. The M.R. of a fibre may be related to the way in which it will hold a molecule such as permethrin. A high M.R. indicates that the pyrethroid may be absorbed within the fibre and thus not be accessible to the insect.

However, the bioassay results for Terylene do not fit this simple model (see below).

2.1.4.2 Crystallinity of fibres

On the basis of the most widely accepted model of fibre structure, all fibres may be considered to consist of two phases, an amorphous (non-crystalline) phase and a crystalline (morphous) phase. Differences in accessibility of fibres have been shown by immersing them in different alcohols and other liquids: as the size of the molecules increased, the penetration decreased. The rate of hydrolysis of crystalline reagents have been used as a measure of the quantity of amorphous material present (Morton & Hearle, 1975).

The cotton fibre, like other vegetable substances, is porous. Part of its porosity is a consequence of the amorphous regions in the fine structure. Also, as the fibre

Table 2.1(b)**Moisture regain of fibres.***

Fibre	Moisture Regain (%)
Cotton	7 - 8
Nylon	3.5 - 5.5
Terylene	0.4
Polypropylene	<0.1

Table 2.1 (c)**Crystallinity of fibres***

Fibre	Crystallinity (%)
Polypropylene	55 - 56
Terylene	55
Nylon	50
Cotton	25

* Data from Morton and Hearle (1975) and Peters (1985).

grows, pores, capillaries and interstices are formed. Calculations assuming an elliptical fibre cross section suggest that about 30% of the total fibre volume is unoccupied space. Cotton can be dyed easily and the colours will often remain fast to repeated washings and prolonged wear. Dyeing is analogous to pyrethroid impregnation and washing experiments indicate that cotton loses less pyrethroid than nylon (Chapter 3).

Pyrethroids are non-ionic which is the optimal characteristic for dyes for hydrophobic fibres e.g. polyesters and polyamides. The particle (dye/pyrethroid) size is extremely important and must be related to formulation (Peters, 1975).

The degree of crystallinity of a fibre depends on a number of factors, one of the most important being the processing to which it is subjected. A rough order of crystallinity of fibres is shown in Table 2.1 (c). This may relate to the uptake and positioning of the pyrethroid and hence its accessibility to the insect.

2.1.4.3 Bonding of fibres

The permethrin molecule (M.W. 391), is equal to or smaller than many of dye molecules. Therefore B.Greenwood (pers.comm.) considers that there is no reason why it should not penetrate the fibres and/or adhere to their surface i.e. permethrin can be thought of as a colourless dye. He also points out that nylon is more amorphous, (less crystalline) than Terylene; therefore the spaces will facilitate easier entry of permethrin into the fibres compared with Terylene. But N.Peacock (pers. comm.) considers that the C=O bond on permethrin might cause attachment to nylon. He also points out that benzene, which is smaller than permethrin, has difficulty in penetrating nylon. Permethrin might therefore have difficulty penetrating nylon and Terylene, as compared to cotton which is much more easily swelled by water (see section on M.R.). A swelling agent such as formic acid might partially dissolve the fibre structure, allowing entry of the pyrethroids. However, if this were done they might then not be accessible to the insects.

Water swells fibres, and in going from the dry to the wet state, nylon and cotton increase in length by 12% and in diameter by 5 & 14% respectively (Lewin and Pearce, 1985). The amount of swelling that occurs depends on the amount of amorphous material and the presence of polar groups. Silks and nylon, for example, have the same amount of amorphous material but there is a larger number of polar groups in silk than nylon so its swelling is much greater.

Since dyes (and presumably pyrethroids) are absorbed exclusively into the amorphous regions of fibres, their availability to insects will depend on the amount of amorphous region - thus cotton's relatively poor performance may be due to the sequestration of pyrethroid into inaccessible regions.

In the dyeing (impregnation) of cotton, first the interchain hydrogen bonding in the accessible regions is disrupted by the water molecules of the dye bath and then the larger dye molecules can penetrate. The dye molecules also form hydrogen bonds (by means of their own reactive substituents) to the cellulose molecules. Because of their size, shape and chemical nature, the dye molecules remain in the fibre on drying (Schick, 1977).

M. Wilding (pers. comm.) suggests that the pyrethroid molecule will probably have some affinity for polyester and cotton and less for nylon, because of the two benzene rings that would form Van der Waal bonds to the benzene rings of polyester and the hydrophobic surface of cellulose.

T. Shaw (pers. comm.) suggests that the reason the nylon performs better than cotton is that the emulsion particles of the E.C. are usually stabilised by negative charge and negative charges will exhaust onto nylon better than cotton.

Laveissière *et al.* (1987) point out that both the fibre and the weave effect the efficiency of screens impregnated with pyrethroids for tsetse control. After impregnation with deltamethrin or alphacypermethrin synthetic fabrics (polyester, acrylic and especially polyamide) caused the highest mortality. A screen made with closely-woven cotton/polyester fabric made of thin thread allowed good fixation of insecticide but prevented tsetse from picking up a lethal dose.

Table 2.1 (a) shows that in the present studies the number of replicates per fibre/pyrethroid varied from the commonly accepted minimum of 3, up to 12 (permethrin/nylon). If the χ^2 test for heterogeneity about the regression line was found to be significant after analysing three replicates, more replicates were carried out, and these were continued until the χ^2 value for all the replicates taken together declined below the critical value for statistical significance.

Cotton required fewer replicates than nylon, except in the case of deltamethrin which required only 3 replicates on both fibres. This may be a clue as to why the toxicity of deltamethrin was similar on both cotton and nylon i.e. deltamethrin may not sink into the cotton fibres as much as do permethrin and lambda-cyhalothrin. The retention of these two pyrethroids on the surface of the nylon fibres may increase their toxicity as well as their irritancy. The latter property may well make it more difficult to carry out repeatable tests and hence have led to the need for more replicates (twice as many replicates were needed on nylon as on cotton).

The easier availability of permethrin on the nylon fibres than on cotton can be visualised from Plate 2. The bioassay data show that, in comparing the three fabrics, nylon recorded the lowest LC_{50} and LC_{90} values for all three insecticides, except in the case of the LC_{50} value for permethrin on Terylene although even here the confidence limits overlap. Hossain *et al.*, (1989 [a]) found the LC_{50} value of permethrin impregnated cotton nets to be about three times as great as that of permethrin impregnated nylon nets.

As already mentioned, the difference between cotton and nylon with deltamethrin was not significant (confidence limits overlap). It is noteworthy that Hervy and Sales (1980) and Wu Neng (cited in Curtis *et al.* 1989) reported that, (based on bioassays with prolonged exposures), deltamethrin on cotton had a slight advantage over deltamethrin on nylon. Where this insecticide is being used (as in China) and where locally produced cotton nets are in widespread use, there is no case for attempting to switch to the use of nylon nets.

Although lambdacyhalothrin and deltamethrin performed similarly on cotton and Terylene, lambdacyhalothrin was markedly more active on nylon than deltamethrin. Where a malaria control programme introduces the use of bednets impregnated with permethrin or lambdacyhalothrin, the fibre of choice should be nylon.

Terylene (polyester) recorded the highest LC_{50} and LC_{90} values of the three fibres for deltamethrin and lambdacyhalothrin. This may be due to a bonding interaction between the alpha cyano-pyrethroids and Terylene, which may not occur with permethrin (which is not an alpha cyano-compound). Terylene is a specialised, relatively expensive polyester, subjected to many finishes (e.g. optical brighteners) which may modify its pyrethroid absorption/binding characteristics and affect its performance.

Comparison of the two alpha cyano-pyrethroids with permethrin shows very large differences in efficacy. It is generally considered that deltamethrin and lambdacyhalothrin are about 10 times more biologically active than permethrin. However, in the present study lambdacyhalothrin ranged from 28 times more effective than permethrin at the LC_{90} level on Terylene to 655 times more effective at the LC_{50} level on nylon. Similarly, deltamethrin showed 8 times greater toxicity at the LC_{90} level on Terylene and 393 times greater toxicity at the LC_{50} level on cotton. Previous comparisons of pyrethroids have generally been on filter paper, mud walls, by fogging, aerosols or topical application. Filter paper and mud may absorb the pyrethroids in a similar manner to that proposed for cotton. This might account for its consistently low performance as compared to nylon. As Whickham *et al.* (1974) emphasize, the mode of pick-up of insecticide is a cause of variation in the results of different test methods. Chadwick (1985) comments that the literature shows many different test conditions with a corresponding wide range of mortality values so that data are difficult to interpret or reconcile.

A comparison of different surfaces and E.C. and wettable powder (W.P.) formulations shows that surface and formulation both greatly affect uptake and

mortality. For example, with permethrin E.C. (200 mg/m²) male Blattella germanica take up 2150 ng of permethrin on glass with a corresponding 100% mortality whereas on plaster they take up 6 ng causing no mortality (Chadwick 1985). This seems to be somewhat analogous to the situation of pyrethroid impregnated nylon and cotton fibres.

2.2 Efficacy of pyrethroid treated polypropylene

2.2.1 INTRODUCTION

In countries where bednets are too expensive to be used by the local population there is increasing interest in the use of other locally available materials, impregnated with pyrethroids but used as curtains. For example, in Tanzania, permethrin treated polypropylene sacks (known as "safleti" because they are initially used for ammonium sulphate fertilizer) have been used as curtains around beds. In Brazil, polypropylene sheeting impregnated with deltamethrin or lambdacyhalothrin has been used in Brazil to create artificial walls for temporary miners' shelters.

The definition of polypropylene (P.P.) is "fibre composed of linear macromolecules of aliphatic saturated hydrocarbon where one carbon atom in two carries a methyl side chain, generally in an isotactic disposition and without further substitution" (see Appendix 2.1).

P.P. is a white semi-crystalline polymer. The degree of crystallinity depends on processing conditions, but is usually between 55 & 65%.

Although there have been several dyeable forms of P.P. offered commercially, P.P. fibres are not usually dyeable or easily impregnated with pyrethroid since the resin does not contain a dyesite, as do other commercially useful fibres. A polypropylene cannot be dyed because it has no polar groups and therefore contains no dye sites capable of reacting permanently with dye molecules. Coloured P.P. fibres are made by adding pigments prior to extrusion (Lewin & Pearce, 1985).

In the following study, permethrin treated "safleti" strands and lambdacyhalothrin treated wide mesh netting were bioassayed over the course of 16 months to assess biological activity. Polypropylene from China was treated with permethrin, deltamethrin or lambdacyhalothrin and bioassayed to test its efficacy.

2.2.2 MATERIALS AND METHODS

The safleti was received from Tanzania already treated at 200mg/m^2 permethrin ("Peripel" 20 % E.C., Wellcome).

The polypropylene from Brazil was impregnated at 5mg/m^2 lambdacyhalothrin.

The polypropylene from China was impregnated (as explained in 2.1) with the following pyrethroids and deposit densities :

permethrin: 800, 200 and 25mg/m^2 ;

deltamethrin: 12, 3.0, 0.04mg/m^2 ;

lambdacyhalothrin: 12, 3.0, 0.04mg/m^2 .

Samples of each were dipped in water to provide controls.

The treated fibres were left to dry for 24 hours and then they were firmly fixed to cork tiles using drawing pins. The tiles were leant against a wall so that the treated fibres were vertical. A WHO plastic bioassay cone was secured to each tile over the fibre and 10 *An.gambiae* were introduced into each cone and into cones covering untreated fibre. After 3 minutes the mosquitoes were removed and placed in paper cups with a pad of cotton wool soaked in 10% glucose solution on top of the nylon netting covering the cups. Mortality was recorded after 24 hours. The tests were carried out at $24^\circ\text{C} \pm 2^\circ\text{C}$ and a R.H. of $64\% \pm 2\%$.

2.2.3 RESULTS

From Figure 2.2 it can be seen that after 4 months the toxicity of the permethrin treated polypropylene begins to decline appreciably. The lambdacyhalothrin treated polypropylene was still causing 100% mortality 16 months

after impregnation.

Table 2.2 shows mortality with 95% confidence limits of Anopheles gambiae after exposure to the treated Chinese polypropylene. The confidence limits of the mortalities associated with the two alpha cyano-pyrethroids overlapped. A comparison of the deposit densities of permethrin and the alpha cyano-pyrethroids which caused equivalent mortalities showed 17 to 625 fold differences.

Untreated polypropylene fibres (from Tanzania) are shown in Plate 1.

2.2.4 DISCUSSION

The P.P. impregnated with lambdacyhalothrin showed excellent kill many months after treatment and this is probably because the lambdacyhalothrin was coating the surface. Thus, for this kind of fibre "impregnated" may be a misnomer. The activity of the permethrin treated safleti fibres was found to decline quite substantially after two months. The reason for this may be that the permethrin which is more volatile than lambdacyhalothrin evaporated and, since there was no replenishment from the interior of the polypropylene, the toxicity of the permethrin treated polypropylene deteriorated quickly.

Barakat et al. (1987) studied the persistence and biological activity of permethrin E.C. on jute and woven polypropylene sheets. They found by G.L.C. that insecticide residues were of shorter persistence on jute than on polypropylene but higher residues were found in grain stored under jute. Bioassays on Tribolium confusum indicated that the deposits of insecticide on the polypropylene were more effective in killing T.confusum than those on jute.

The fibres tested could be used in a number of ways and are a promising alternative to bednets especially where nets are not affordable or are disliked because they reduce the circulation of air.

Treated fibres could be placed around the eaves, doors and windows of houses. In an experiment in Tanzania, the safleti was hung around the bed in a loose

curtain about 70 cm high. The safleti fertilizer bags are washed after use and sold for the equivalent of about 20-30 U.S. cents each. Approximately 3 are required to go around an ordinary bed (T.J.Wilkes pers comm.). Since bednets cost about one month's minimum wage, safleti would appear to be a practical alternative.

About 16 km of treated polypropylene are reported to have been wrapped around the temporary shelters of gold miners in Brazil (R. Webb, pers.comm). It is not known if rainfall reduces the effectiveness of treated polypropylene placed around the gold miners' dwellings.

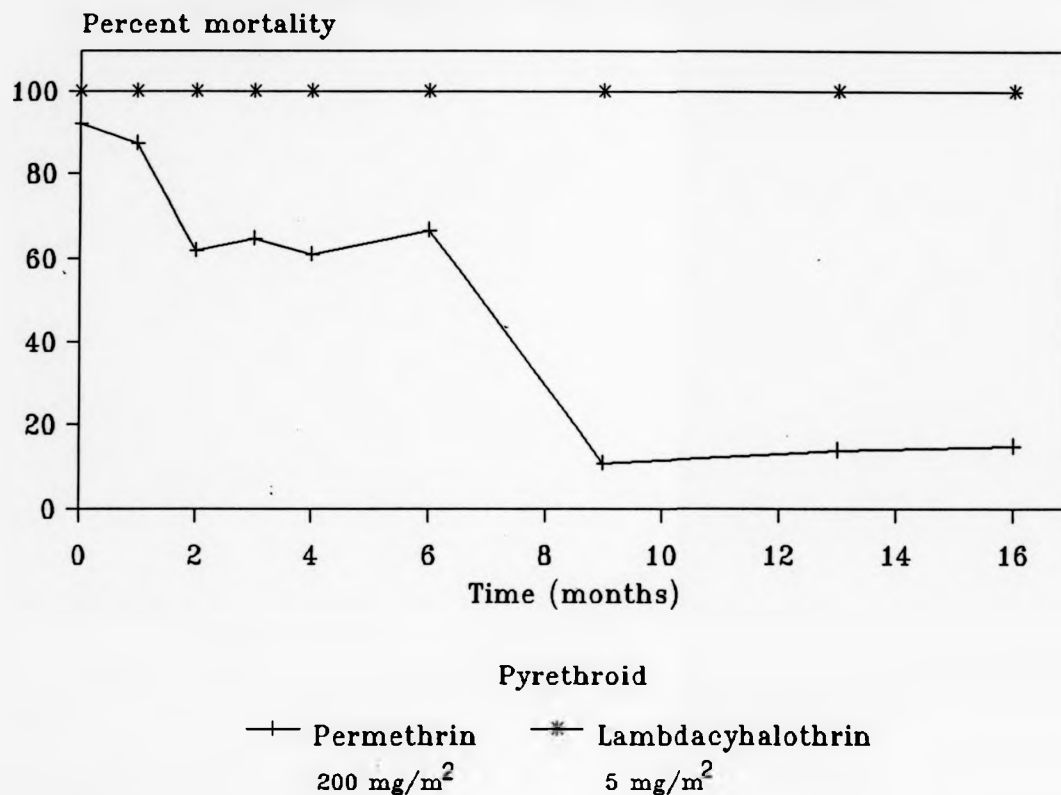
When a polypropylene curtain became dirty one would probably replace it with a new impregnated curtain rather than attempting to wash it. Thus wash-fastness is not an important consideration.

Polypropylene is an ubiquitous fibre. Cheap bags made of it can be obtained world-wide in one form or another. In many countries, including China, Thailand and Turkey large, extremely cheap holdalls or carrier bags are available and these could be cut up and treated.

Because P.P. fibre producers are now able to make a large variety of products and the textile market has learned how to use these products better, P.P. fibres have been able to achieve an increasing share of the textile market and are expected to occupy an even greater part of the market in the future.

	1971	1987
Share of total fibre market	3%	10%

Figure 2.2 Mortality of *Anopheles gambiae* after exposure to pyrethroid treated polypropylene at intervals over 16 months



Means of 3 replicates
(N=45-52)

95% c.l. on control mortality = 1-7%

Table 2.2

Mortality of *Anopheles gambiae* after 3 minute exposure to pyrethroid treated polypropylene from China.

Pyrethroid/ Target deposit density mg/m ²	Percent Mortality*	95% Confidence Limits **
Permethrin		
800	100	89.1 - 100
200	60.6	42.1 - 77.1
25	25.7	12.5 - 43.3
Deltamethrin		
12	88.6	73.3 - 96.8
3.0	79.4	62.1 - 91.3
0.04	25.0	11.5 - 43.4
Lambdacyhalothrin		
12	100	88.8 - 100
3.0	91.4	76.9 - 98.2
0.04	40.6	23.7 - 59.4
Control	3.0	0.08 - 15.8

* Means of 3 replicates (N = 31-35).

** From binomial tables

SUMMARY of Chapter 2

- (1) The bioassay data (based on 3 minute exposures) showed that the LC_{50} and LC_{90} values for the 3 pyrethroids were higher on cotton than on nylon, although the confidence limits of deltamethrin on nylon overlapped with those on cotton. The confidence limits of the LC_{50} of permethrin on Terylene overlapped with those on nylon, but the LC_{50} values of deltamethrin and lambdacyhalothrin treated Terylene were higher than those on nylon.
- (2) Scanning electron micrographs showed a liquid film of active ingredient visible around the permethrin treated nylon fibres which was not visible around similarly treated cotton fibres.
- (3) Polypropylene fibres treated with lambdacyhalothrin at 5mg/m^2 caused 100% mortality one year after treatment.

Plate 1 Scanning electron micrographs of untreated fibres

(a) Untreated nylon fibres.

Particles of dust (d) are visible.

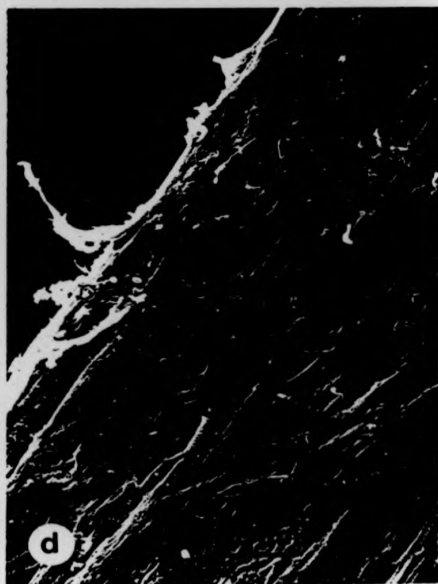
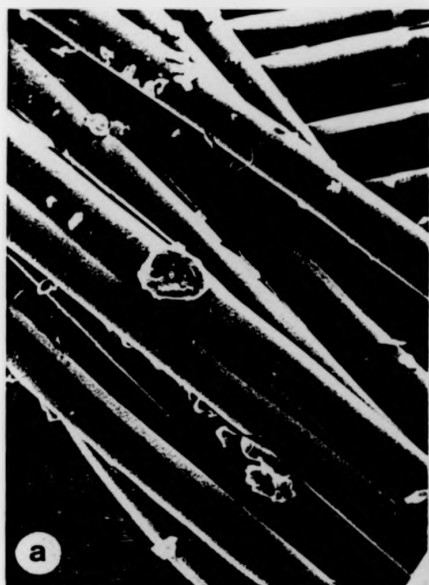
(b) Untreated cotton fibres.

(c) Untreated Polyester fibres.

(d) Untreated polypropylene fibres.

Finishing materials (denoted by arrows) are visible between individual fibres of nylon, cotton and Terylene.

Plate 1



**Plate 2. Scanning electron micrographs of permethrin treated nylon
and cotton fibres and untreated nylon fibres**

(a) Nylon treated at 0.8g permethrin/m².

A liquid residue of permethrin is visible on the fibres.

(b) Cotton treated at 0.8g permethrin/m².

No surface residue is visible.

Surface finish is visible (see arrow).

(c) Nylon treated with blank E.C. at 0.8g/m² equivalent.

Surface finish is visible (see arrow).

(d) Nylon dipped in water.

Surface finish is visible (see arrow).

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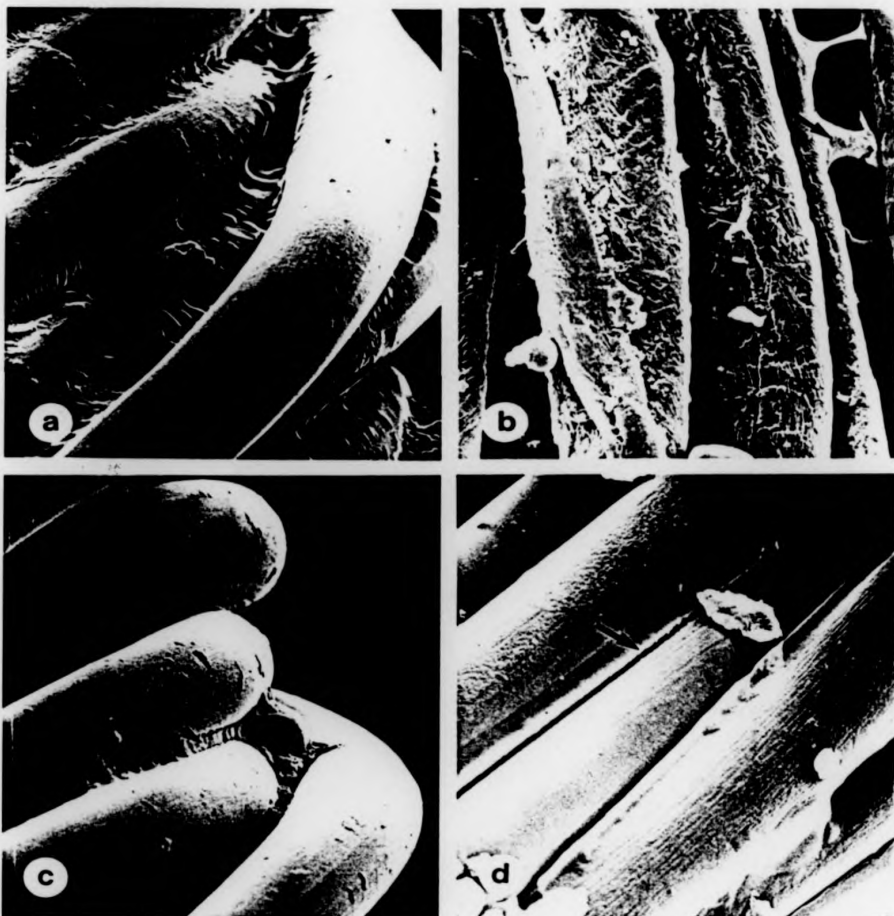
(c) Nylon treated with blank E.C. at 0.8g/m² equivalent.

Surface finish is visible (see arrow).

(d) Nylon dipped in water.

Surface finish is visible (see arrow).

Plate 2



CHAPTER 3

STUDIES ON STANDARD FORMULATIONS, THE EFFECT OF WASHING ON IMPREGNATED FABRICS AND THE DEVELOPMENT OF A WASH-RESISTANT FORMULATION

3.1 INTRODUCTION

Nowadays the range of pyrethroids is bewilderingly large and formulation has advanced a long way from the old practice of making a solution in oil (Chadwick, 1985).

Emulsifiable concentrates (E.C.s) and wettable powders (W.P.s) are the only formulations which have been used for the impregnation of bednets and other fabrics to date. Effects of raising the temperature of the emulsion and addition of wetting agents and acids to the emulsion have been reported by Lindsay *et al.* (in press).

The type of formulation required depends predominantly on 5 factors:

- (i) Biological activity and mode of action,
- (ii) Physical properties including persistence,
- (iii) Chemical properties,
- (iv) Method of application,
- (v) Cost.

The E.C. is a relatively easy formulation to handle in the field and generally has a highly cost efficient performance which probably accounts for its popularity for the impregnation of bednets. It is prepared by dissolving or mixing the insecticide with a water immiscible solvent and suitable emulsifying agents. The emulsifiers promote the spontaneous dispersion of the active ingredient (a.i.) and solvents which are lipophilic in water. The solvents are usually aromatic hydrocarbons which are readily available and cost effective. However, E.C.s are not as cheap as W.P.s or suspension concentrates (S.C.s) and are prone to cause phytotoxicity in agricultural use because the active ingredient is in its molecular form (i.e. the a.i. is completely miscible in the solvent(s)) and the solvents and emulsifiers have phytotoxic properties and can have synergistic effects when added to water.

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Oil E.C.s incorporate an oil, usually white oil (specific fraction from a petroleum feedstock) into the formulation. This improves operator safety, e.g. reducing possible skin /eye irritation by decreasing the amount of solvent present.

The W.P. is prepared by mixing the insecticide, a dispersing agent, wetting agent and filler (normally a clay), grinding in a pin or hammer mill and mixing again to give a homogeneous product. W.P.s are the commonest type of formulation and have dominated the public health market for insect vector control especially residual house spraying (WHO, 1982).

An Oil in Water Emulsion (E.W.) is made by emulsifying oil into water, which is accomplished by the addition of small quantities of emulsifier whilst mechanically agitating with a high speed homogeniser. These formulations have the benefit of being diluted already, therefore they are safer to handle in the field than E.C.s for example, and are less phytotoxic and toxic to the skin.

Micro-encapsulated formulations (C.S.) are prepared by interfacial polymerisation. An oil phase containing the pesticide and reactive monomer is emulsified in water. A second monomer is then added and the two co-polymerise at the oil and water interface, forming an insoluble polymeric shell around the droplets of pesticide. C.S. formulations can be diluted in water for use as a spray or can be used neat as seed treatments. The capsule wall modifies the availability of the pesticide and can give increased persistence, reduced toxicity (especially dermal/vapour) and reduced phytotoxicity. The capsules are very small (1-5 microns) and should adhere well to the substrate.

Suspension concentrates (S.C.s) are a suspension of a solid pesticide in a liquid, normally water. They are difficult formulations to perfect, the particles may sediment and eventually form a thick clay which is difficult to re-suspend. They should be diluted with water.

As explained in the introduction (1.14), washing of nets can be a serious problem since it removes a considerable amount of pyrethroid from impregnated

fabrics. Washing is the main cause of pyrethroid loss from bednets, although small amounts may be lost by abrasion (e.g. Darriet *et al.*, 1984) and possibly by cutaneous absorption. It is not yet clear whether the losses during washing are due to the chemical action of the detergent, physical abrasion of hand/machine or a combination of both factors.

The aims of the following studies were:

- (1) To compare initial toxicity, toxicity at intervals over one year and toxicity after washing of a number of different formulations of lambdacyhalothrin and the W.P.s of permethrin, deltamethrin and lambdacyhalothrin;
- (2) To assess the effect of (a) soap/physical abrasion and water, (b) physical abrasion and water only and (c) water only on pyrethroid loss.
- (3) To assess the effect of soap on subsequent loading of pyrethroid, i.e. to determine whether washing reduced pyrethroid uptake;
- (4) To assess the effect of addition of acid and elevated emulsion temperature on pyrethroid uptake by fibres and subsequent wash resistance;
- (5) To determine whether the time spent in the emulsion affected pyrethroid uptake by the material, or caused an increased retention of a.i. after washing;
- (6) To synthesize a formulation that reduced the loss of a.i. on washing, i.e. that was more wash-resistant.

3.2 METHODS AND MATERIALS

3.2.1 Standard Formulations

The formulations tested were the Oil E.C. (2.5%), E.W. (5.0%), W.P. (10.0%), C.S. (10.0%) and S.C. (7.5%) of lambdacyhalothrin and the W.P. of permethrin (25%) and deltamethrin (2.5%). Impregnation of cotton and nylon fibres was carried out as explained in Chapter 2, the only difference being that the W.P.s and S.C.s were mixed into a paste with a small amount of water and then diluted with the remainder of the water. Target deposit densities were: lambdacyhalothrin - 6.0 mg/m^2 ; deltamethrin W.P - 6.0 mg/m^2 ; permethrin on nylon 200 mg/m^2 and permethrin on cotton 800 mg/m^2 . Scanning electron micrographs (section 2.2.6) were taken of the permethrin W.P. treated fibres (Plate 3). Pyrethroid uptake by the fibres was analysed using gas chromatography (Appendix 2.1). Toxicity was assessed using 3 minute bioassays in WHO kits (section 2.2.5). Bioassays were carried out 1 day after impregnation and then 1, 6 and 12 months later for the lambdacyhalothrin formulations and 3 days, 10 days, 1, 2, 3, 6 and 12 months after impregnation for the W.P. treated fibres. Only the formulations showing good agreement between target and actual deposit density, and those it was considered might resist loss of a.i. on washing were tested after washing.

Washing method.

Two pieces (30 x 36 cm) of netting were washed, one at a time, in cold water using "cow fat" soap (from The Gambia) for two minutes and then rinsed in cold water for one minute. Netting was left to dry for 24 hours, after which one piece was taken for bioassay and G.C. analysis (1 Wash), the other piece being subjected to another wash, with bioassays and G.C analyses being carried out when the netting was dry (2 Washes).

3.2.2. Effect of soap

Nylon and cotton netting impregnated at target deposit densities of 400 and 800 mg permethrin a.i./m² respectively was (a) washed with cowfat soap and cold water, (b) cold water only or (c) placed in a bowl of cold water for 3 minutes. The amount of permethrin left was determined by G.C. Bioassays were carried out to assess the toxicity of the netting.

3.2.3 Effect of one wash and reimpregnating

After one wash with water plus "cowfat" soap (from The Gambia) or "Foama" detergent (from Tanzania) and water, or water only, the netting was left to dry for 24 hours and then re-impregnated.

The amount of pyrethroid left after one wash and the amount found on the netting after re-impregnating was determined using gas chromatography. Bioassays were carried out to assess the toxicity of the netting to mosquitoes.

3.2.4 Effect of acid and elevated temperature

The emulsion of permethrin, adjusted to give 400 and 800 mg/m² for nylon and cotton netting respectively, was made acidic (pH 2.5-3.0) using concentrated hydrochloric acid, added one drop at a time using a pasteur pipette and tested with pH paper. When the desired pH was obtained, the emulsion was gently heated to boiling point and the netting was dipped in it, squeezed out and left to dry. Bioassays and G.C. analyses were undertaken of the fibres unwashed, washed once and washed twice.

3.2.5 Effect of time in emulsion

Nylon and cotton netting were left for 10 minutes, 1 hour and 12 hours in emulsions calculated to give 400mg/m² and 800mg/m² respectively assuming passive

uptake of a.i. Samples were bioassayed and chemically assayed before and after washing and compared with the netting dipped for the usual time of about 3 minutes.

3.2.6 Development of wash-resistant formulation

Three approaches were tried in attempts to develop a wash-resistant formulation of permethrin and lambdacyhalothrin:

- (a) dissolving a sticker/film former into the E.C.,
- (b) adding a film former (latex) to the emulsion and then treating the netting,
- (c) Impregnating the netting, allowing it to dry and then treating the netting with the film former (latex).

Further details of these three approaches are as follows:-

- (a) A starting point of 5% polymer in permethrin 25% E.C. was chosen from a pilot study assessing the solubility of the stickers in the solvents of the E.C. 1.44 g of a polymer was added very slowly over a 24 hour period to 27.36 mls of E.C which was warmed gently to about 35°C on a hot plate and stirred continuously using a magnetic stirrer.

The polymers were: "Imprez" 100 (Synthetic resin from I.C.I Specialities, Leatherhead, Surrey, England), "Cereclor" 70 (chlorinated paraffin from I.C.I Specialities), paraffin wax (BDH Chemicals Ltd, Poole, Dorset, England), Shellac (natural resin secreted by the insect Laccifer lacca - order Homoptera, family Coccidae; from BDH), three polystyrenes (molecular weights 100,000, 200,000 and 300,000 from BDH Chemicals Ltd), polyvinyl acetate (PVAc, m.w. 160,000 from BDH Chemicals Ltd), cellulose acetate phthalate (CAP from Kodak Ltd, London) and wood resin (from BDH).

The paraffin wax, PVAc, CAP and wood resin were not sufficiently soluble, when even 1% solutions were attempted over 48 hours. Good

emulsions (as shown by emulsion stability tests, see Appendix 3) of the E.C.s containing the other 6 polymers were obtained. Three different molecular weights of polystyrene, and a range of polystyrene concentrations (0.5, 1.0, 2.5, 5.0 and 10.0%) found 5.0% to be the optimum in the permethrin formulation. Three acrylic resins from Rohm and Haas U.K Ltd, Croyden, England, called "Paraloid" and code numbered B44, B67 and B82 at 1% and 2.5% concentrations and an oil (Aplus 1411F from I.C.I Specialities) were tested in the lambdacyhalothrin formulation to try and improve on the polystyrene formulation.

Three pieces of nylon were impregnated at target deposit densities of 400mg/m². One piece was bioassayed and chemically assayed for pyrethroid content and washing tests were carried out on the other two pieces.

- (b) A 5% latex solution in water was added to the already prepared emulsion before the netting was dipped. 3.1 ml of latex were dissolved in 56.9 ml of emulsion. 2.9 ml of this was added to 59.1 ml of water. The latex formulations tested were R8075/157/1, R8075/157/2 and R8075/157/3 (I.C.I. Paints Division, Slough, Berkshire) and "Vinacryl" 7172, "Vinamul" 18134 and "Vinamul" 18207 (Vinamul Ltd, Mill Lane, Carshalton, Surrey, SM5 2JU).
- (c) The pre-impregnated and dried netting was dipped in a 5% latex solution (1 ml of latex in 19 ml of water), left to dry and then bioassayed and chemically assayed. The latex formulations tested were those listed above.

3.3 RESULTS

In Table 3.1 (a) the dilution details refer to the appearance of the formulation by itself and on addition to water. Gas chromatographic analysis of deposit density, frequently revealed underdosing or overdosing, i.e. less or more than the deposit density expected on the assumption of passive absorption of active ingredient from the weight of liquid taken up. Nylon showed overdosing with all formulations except the oil E.C. (deposit density of 6.0 mg/m^2). Cotton showed underdosing with all formulations except the S.C. where overdosing occurred (14.0 mg/m^2). The W.P.s on nylon showed fairly good agreement with target but this was not the case with the cotton netting (Tables 3.1 (a) and (b)). None of the formulations tested withstood washing better than the E.C. (Table 3.1 (c)). On washing, the cotton netting lost less a.i. than nylon.

Figure 3.1 (a) shows bioassay mortality of Anopheles gambiae due to the lambdacyhalothrin formulations. Figure 3.1 (b) shows the bioassay mortality of the three W.P.s on nylon and cotton at intervals over one year. Figure 3.1 (c) shows initial mortality due to the E.C.s on nylon and cotton and mortality at 6 months and one year after impregnation. The lambdacyhalothrin S.C. on nylon gave the highest mortality in the test after one year, although its 95% confidence limits overlapped with those for the E.W. on nylon. The lambdacyhalothrin W.P. on cotton was not significantly better than the same formulation on nylon after one year, but both were better than the 2 other W.P.s on nylon or cotton. The E.C. of lambdacyhalothrin on either cotton and nylon was not significantly different from the S.C. or the E.W. after one year. Both permethrin and lambdacyhalothrin did not appear to lose activity as quickly on cotton as they did on nylon.

The effect of soap washing on the retention of permethrin is shown in Table 3.2. With nylon, the first wash with water and rubbing removed the same amount of permethrin as the first wash with soap, water and rubbing, with a consequent lowering of bioassay mortality. Immersion of the treated nylon in a bowl of cold water

removed a great deal of permethrin but not as much as soap and rubbing did. The deposit densities in the cotton were not much affected by either type of washing but the corresponding bioassay mortalities were very low despite the high retention of a.i.

The effect of soap and detergent on subsequent uptake of pyrethroid is shown in Table 3.3. One wash, using either soap or detergent, reduced the subsequent uptake of permethrin, as shown by G.C. analysis but the confidence limits of mortality after bioassay on the netting overlapped with the bioassay mortality of the netting washed in water only.

Table 3.4 shows that the addition of acid to the emulsion and impregnation at elevated temperatures affected permethrin uptake by nylon at the higher deposit density (400mg/m^2), but these treatments did not increase the washfastness of the netting. The uptake of permethrin by cotton at both deposit densities was increased by acid and elevated temperatures, but again this did not cause the fibres to retain the permethrin on washing.

There was no effect on permethrin uptake and washfastness of the nylon netting due to increasing the time spent in the emulsion. The cotton netting which spent 12 hours in the emulsion showed an increased deposit density although it did not show any better permethrin retention on washing than the permethrin which spent the usual 3 minutes in the dip (Table 3.5)

Tables 3.6 (a), (b) and (c) show the results of the permethrin plus polymer, lambdacyhalothrin plus polymer/oil and permethrin plus latex formulations respectively. From 3.6 (a) it can be seen that 5% polystyrene (m.w. 100,000) showed the best combination of permethrin target deposit density, retention of a.i. and bioassay mortality after washing. Increasing the molecular weight did not have an effect on retention of a.i. and in fact the netting samples impregnated with the two higher molecular weight polystyrenes were associated with significantly lower bioassay mortalities than the 100,000 m.w. polystyrene after 2 washes. Both 10% polystyrene and the latex formulations tested caused masking of the a.i., i.e. poor bioassay results compared with what might be expected from the G.C. result. The

addition of oil to the lambdacyhalothrin formulation did not reduce the loss of a.i. on washing the netting (as assessed using G.C.) but appeared to improve the performance in the bioassay of the available a.i. Plate 3 shows scanning electron micrographs of nylon and cotton fibres treated with wettable powder and wash-resistant formulations of permethrin.

3.4 DISCUSSION

As already pointed out, with E.C. formulations the absorption of insecticide from an aqueous dip bath onto fabric is generally proportional to the concentration of insecticide in the bath and to the absorptive capacity of the fabric (Hossain and Curtis, 1989 [a]). However, with many of the formulations tested, the chemical assays revealed the actual deposit densities to be very different from the target ones, implying more complex interactions between the fabrics and pyrethroids than those that occur with the E.C.s.

Some of these formulations would be difficult to handle in the field. For example, the S.C. was a thick viscous paste which was difficult to measure out - a disadvantage in the field where simple, quick delivery of insecticide is desirable. Laveissière *et al.* (1986) found that a U.L.V. formulation of deltamethrin was as good as the E.C. for treating tsetse screens, but they point out that the requirement of the U.L.V. for a solvent different from water would prejudice its use in campaigns carried out by rural communities. Similarly, the W.P. showed good results on polyester netting but they felt that handling and titrating it in the field would be impracticable. Formulation can modify the nature of the deposit, thus affecting the performance of the insecticide. Formulation affects the following:

- (a) the distribution of insecticide over the surface of the fabric,
- (b) penetration into the fibre,
- (c) the transfer of insecticide from fibre to insect,
- (d) the persistence (rate of loss of a.i.).

The bioassays of the W.P.s of deltamethrin and permethrin showed a marked loss of toxicity soon after impregnation. However, the lambdacyhalothrin W.P. was still killing mosquitoes quite well one year after impregnation. The W.P. of deltamethrin has been used on 2.4 million nets in Sichuan Province, China, at a target deposit density of 10 mg/m^2 (Curtis *et al.*, 1989).

In the present studies, the permethrin W.P. was less active and less persistent than the E.C. The scanning electron micrographs (plate 3) show the W.P. deposit to consist of scattered clumps of filler (e.g. diatomaceous earth) containing adsorbed permethrin. The particle sizes are large ($1\text{--}30 \mu\text{m}$), adhesion is poor and coverage is low and patchy. In contrast, the E.C. covers the nylon fibres evenly, with good adherence (plate 2). Thus it would seem that mosquitoes are more likely to contact the a.i. on fibres treated with the E.C. than with the W.P. The cotton netting treated with the E.C.s of permethrin and lambdacyhalothrin lost its toxicity to mosquitoes less rapidly than the nylon E.C. treated netting. Barlow and Hadaway (1968) compared the loss of a deposit of arpopcarb from filter paper or glass, which may analogous to the loss of pyrethroids from cotton or nylon. On glass, the rate of loss of insecticide was linear, whereas on the filter paper, after a short period of linear loss, the rate decreased very sharply. The time during which the insecticide evaporates rapidly, and with zero order kinetics corresponds to the period of bioassay when no change in toxicity occurs (which corresponds to approximately the first month - see figure 3.1 (c)). It appears that the insecticide is then being lost from a layer which is essentially pure insecticide and so its properties are not dependent on concentration. Once this external portion has gone, the remainder of insecticide is presumed to be within the fibres and toxicity is dependent on diffusion of the insecticide out of the fibres. With treated nylon, the majority of pyrethroid may be on the surface, so that when this has gone, there is very little pyrethroid internally to replace it.

After washing, none of the formulations tested retained more a.i. than the E.C. Because of differential loading of a.i. on to the fibres, comparison of the different

unwashed lambdacyhalothrin formulations over time is difficult. However, the S.C. on nylon was killing well after one year and an initial loading of about 11mg/m^2 . The S.C. used in these studies was prepared by absorbing liquid lambdacyhalothrin on to a solid carrier dispersed in water. This produces a "mixed" system with some a.i. absorbed onto the solid but some also emulsified into the water. The S.C. therefore comprised both a particulate deposit of a.i. and a liquid deposit. The former was probably responsible for the high toxicity after one year. Similarly, the lambdacyhalothrin W.P. was still causing high rates of mosquito mortality one year after impregnation.

The first time that the nylon netting was washed with water only, the same amount of a.i. was removed as with the soap and water wash. This probably corresponded to the removal of the surface coat, because the second wash with water only did not cause such a large percentage depletion. There was a considerable loss of a.i. when the treated nylon was left in a bowl of water, which is contrary to what one might expect since permethrin is insoluble in water and hydrophobic. The explanation is probably that residual emulsifier on the netting causes the permethrin to come off. The G.C. results show that the amount of a.i. in and on the cotton was hardly affected by any washing regime. The obvious inference that the a.i. is bound internally is corroborated by the low bioassay mortalities.

Analysis of deposit densities on both cotton and nylon netting, after one wash using "cowfat" soap or "Foama" detergent and re-impregnation showed a reduced loading of permethrin compared to that on netting washed in water only. The reason for this is probably that the soap/detergent leaves a residual deposit which interferes with permethrin uptake by the fibres when the nets are dipped. In several field trials householders have been advised to wash their nets just prior to re-impregnation, in the hope that they will not then wash them again for several months. However, several householders in Tanzania commented (C.Curtis and T.Wilkes, pers. comm.) that the washed and re-impregnated nets were not killing so many mosquitoes as the nets did when initially impregnated. The G.C. results in Table 3.3 give some support to these

comments but the bioassay mortality did not differ significantly between re-impregnated samples which had been washed with water only, or soap and water.

Making the emulsion acidic (pH 3) and increasing its temperature caused increased pick-up of permethrin by the cotton and nylon fibres. Neither retained the permethrin better on washing than the netting treated at ambient temperature (about 20°C). In fact, with the cotton netting a 40% loss of the initial deposit density was recorded on washing whereas the cotton treated at ambient temperatures did not lose any permethrin on washing. The permethrin lost was probably not bound internally but was more superficial. The higher bioassay mortality results before washing also indicate that this was the case.

The use of pH 3.2 at 97°C was found to increase the uptake of permethrin by polyester netting in trials in The Gambia (Lindsay *et al.*, in press and data in Curtis *et al.*, 1989). The deposit densities were found to be 2-3 times more than the deposit densities expected on the assumption of passive uptake. The treatment caused about 60% retention of a.i. after washing compared with 40% on/in fibres treated at neutral pH and ambient temperatures with target deposit densities of 500 mg/m².

Possible explanations for the differences in the results in the present study and the Gambian study are that, in the latter, the netting was polyester and was left to cool for 5 minutes before wringing out, whereas in the present study, the netting was nylon and was wrung out immediately.

Decreasing the pH, by addition of acid, is thought to cause more rapid movement of the permethrin molecules into the fibres due to change in chemical potential (B.Greenwood, pers. comm.). Heating may make the permethrin emulsion unstable so that the permethrin, which is hydrophobic, preferentially adheres to fibres. Acid and heat treatment can cause fibres to swell, as does mercerization of cotton, which consists of treatment with aqueous sodium hydroxide. Concentrations of 14-32% NaOH increase the cross-sectional areas by up to 80% and cause contraction of the fibre length by as much as 19% of the original. The phenomenon is dependent on

temperature and concentration. The change in lattice type is accompanied by increase in accessibility, shown by increased moisture regain, and greater dye uptake giving deeper shades of dying. Reactants which proceed into the crystalline regions are called swelling agents and include anhydrous liquid ammonia and strong acids. However, it might be dangerous for villagers to use strong acids or alkalis and heat treatments would use up valuable fuel.

Acid and heat treatments also cause selective removal of permethrin from the emulsion and the concentration of permethrin in the emulsion would have to be repeatedly replenished. This problem does not occur with treatment with E.C.s at ambient temperature and neutral pH. If stronger evidence of the advantages of acid and heat treatment for bed nets emerged it could be carried out in a factory. Factory treatment of netting has the advantage of controlled conditions which cause more uniform loading of pyrethroid. Analysis of random samples from a 200 metre roll of polyester treated in a factory showed a mean loading of 420mg/m^2 (with standard deviation of 33mg/m^2) from a target of 400mg/m^2 (K.Dorgan, pers. comm.). In Chapter 6, the variation in deposit densities from samples from nets dipped by hand is discussed.

Another advantage of factory treatment is that insecticides which are potentially more toxic or more unpleasant to handle as E.C.s (e.g the alpha cyano-pyrethroids such as lambda-cyhalothrin and deltamethrin) could be more safely used.

Factory treatment alone without retreatment in the village might be used if it was decided to use cheap netting of low durability and if regular washing of nets was not the local custom. The nets might be disposed of after one year and adequate insecticidal effect of lambda-cyhalothrin could be expected over this period (e.g Figure 2.2). However, where more durable nets are preferred and/or where washing is common, re-treatment in the village with a benign compound such as permethrin would seem to be the best policy.

It may be that drying at elevated temperatures bonds the pyrethroid more strongly to the netting, making the pyrethroid deposit more washfast. Moth-proofing

agents applied to dry spun yarns with the spinning lubricant, result in the moth proofing agent being only loosely bound to the fibre surface but a firmer bond is achieved by steam treatment. (I. Holme, pers. comm.).

Tightly bonded pyrethroid would be effective in killing Clothes Moth Larvae (*Tineola bisselliella*) and Carpet Beetles (*Anthrenus flavipes*) which eat fibres. However, such bonded pyrethroid would probably not kill mosquitoes which may only touch a net briefly.

Time spent in the emulsion by the netting did not have an important effect. Only the cotton netting that spent 12 hours in the emulsion showed increased uptake of permethrin (about 90% more compared to the usual dip deposit density), but it did not hold onto this when washed. Overnight impregnation would not be popular because people like to have their nets back to use on the night of treatment.

To increase the effectiveness of cotton, one could try mixing the E.C. with something which would prevent it being absorbed into the fibres. Gerolt (1957) points out that a promising line of approach for the prolongation of insecticide persistence on surfaces such as mud panels would be the combination of the insecticide with certain types of non-sorbable material, e.g. resin. This would act as a physical barrier between the insecticide and the sorbent - in this case cotton. Thus, a wash-resistant formulation might also increase the initial toxicity of permethrin on cotton. The scanning electron micrographs (Plate 3) of the nylon and cotton samples treated with the wash-resistant formulation of permethrin shows a surface deposit on both types of fibre. This contrasts with the micrographs of the nylon and cotton fibres treated with ordinary permethrin E.C. where a surface deposit is visible on the nylon but not on the cotton.

The synthesis of a wash-resistant formulation was problematic. There is a very delicate balance between keeping the a.i. on the net after washing but also making it available for pick-up by the mosquito through its tarsi. The polymer latex dispersion added to the emulsion caused an excellent retention of a.i. (e.g. 85% after 2 washes - latex R8075/157/2) but the corresponding mosquito mortality was very low,

suggesting that the latex was either binding the a.i. too strongly to the fabric or covering it and thus preventing it from being picked up by the mosquitoes. Many of the polymers tested caused masking of the a.i. prior to washing and the pyrethroid actually performed better after washing.

Incorporation of an oil into the formulation did not improve washfastness but toxicity was very good after two washes. Adams *et al.* (1988) found that the addition of a surfactant to a permethrin formulation increased its efficacy on leaves in a field trial ten-fold. They did not attribute this to droplet spread on the leaf surface, or to differences in the surface tensions of the mixtures. Instead they suggest that the increased efficacy may be due to an improvement in the way the a.i. diffused from the oil droplet into the surface waxes, or possibly enhancement of the mobility of the pyrethroid towards the active site once it enters the insect. The oil on the fibre may be providing a similar surface, facilitating pick-up of a.i. so that the amount available is optimized.

Spraying has been advocated for the treatment of bednets. Water soluble pesticides can be formulated as a water in oil suspension for ultra low volume (U.L.V.) or electrodyne (E.D.) applications. U.L.V. formulations are prepared by dissolving, emulsifying or suspending the active ingredient in solvents of low volatility. However, the formulations are usually diluted in diesel and/or kerosene which makes them pungent and highly flammable - unacceptable characteristics for bednet application. There are aqueous formulations but these are expensive and have very similar properties to E.C.s.

Electrodynamic formulations are very similar to U.L.V. formulations but incorporate specific electrical properties. Both U.L.V. and E.D. formulations require spraying equipment and trained operators for application. This would be a drawback to their use, unless spraying equipment and operators are already available from house spraying or agricultural insect control programmes. One of the great attractions of dipping nets is that it does not require equipment and trained personnel, unlike residual house-spraying programmes.

A further disadvantage of spraying nets is that much insecticide passes through the net and is wasted, as shown in the spraying of netting screens (= targets) for use against tsetse (S.Torr, pers. comm.).

3.5 SUMMARY of Chapter 3

- (1) Various types of formulations were tested for initial toxicity, toxicity during one year's storage and after washing. Only one was found to perform significantly better after washing than the ordinary E.C.s .
- (2) This formulation which showed increased resistance to loss of a.i. and insecticidal power, on washing was made by incorporating polystyrene into the E.C.
- (3) Physical abrasion was found to be as important as soap in the removal of the a.i. from nylon fibres during washing.
- (4) Acid and elevated bath temperature increased the uptake of permethrin by cotton and nylon fibres but did not make them more resistant to loss of a.i.
- (5) Cotton netting which spent 12 hours in the emulsion bath showed increased uptake of permethrin but no resistance to loss of a.i. on washing.

Figure 3.1 (a) Mortality of *An.gambiae* in tests conducted periodically over one year on nylon and cotton netting treated with formulations of lambdacyhalothrin

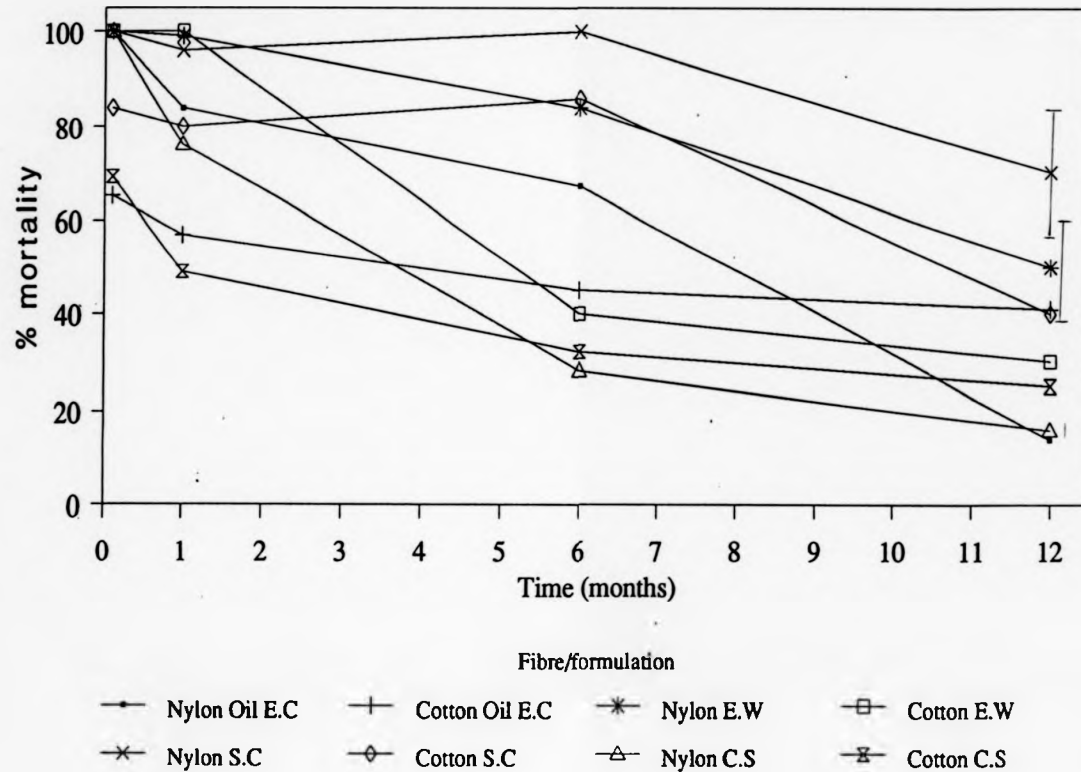
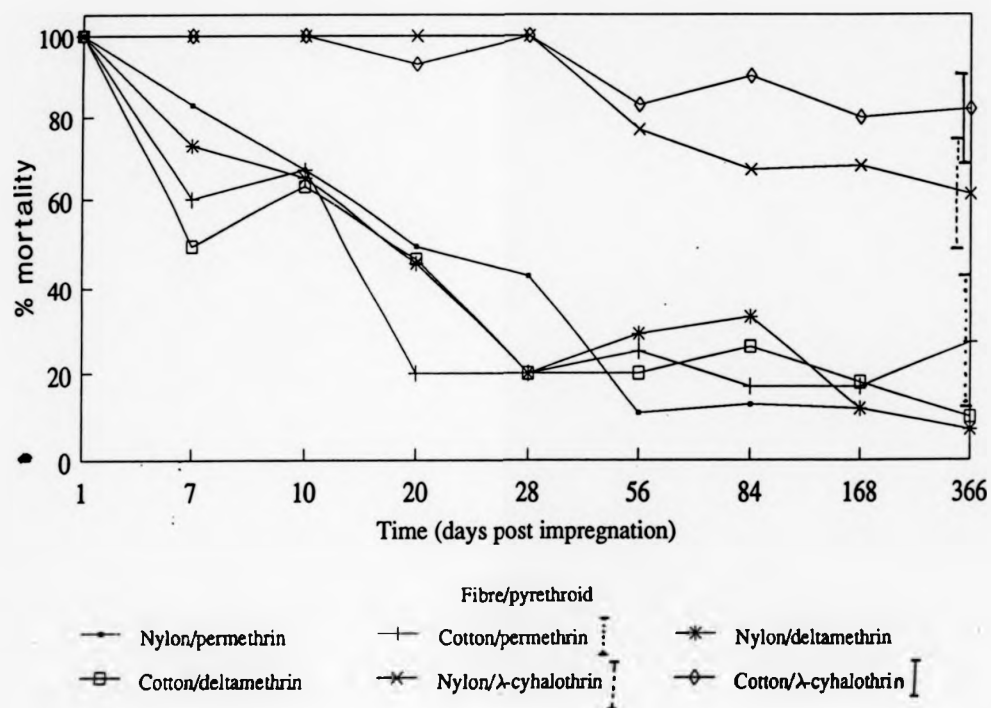


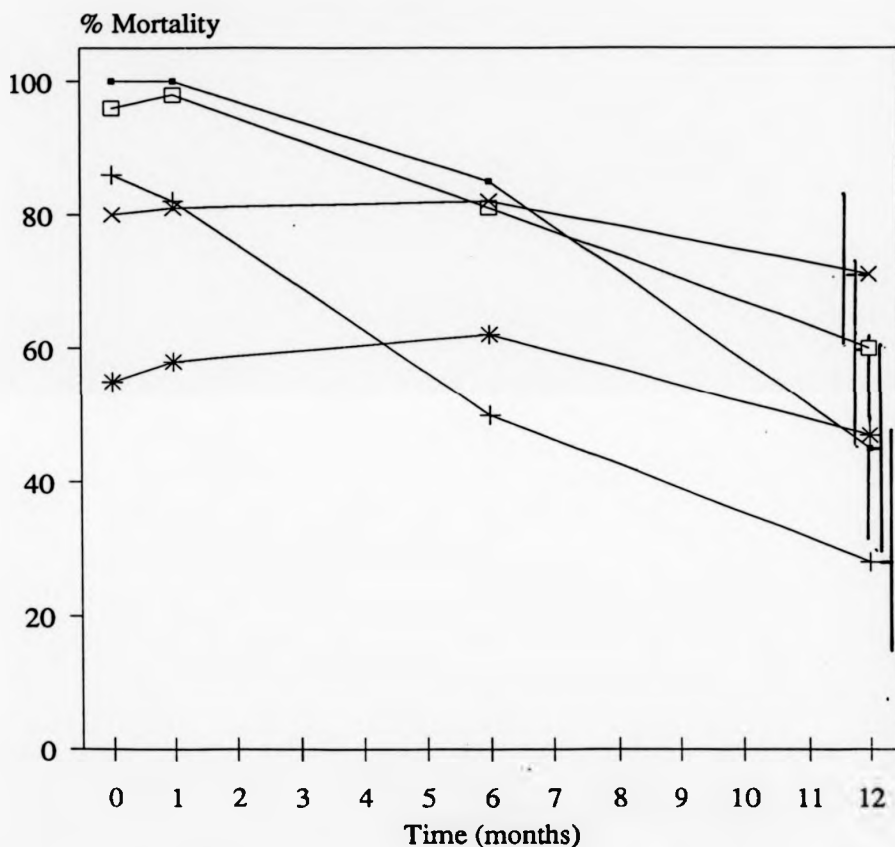
Figure 3.1 (b) Mortality of An.gambiae in tests conducted periodically over one year on nylon and cotton netting treated with wettable powder formulations



Means of 3 replicates

Bars at 366 days = 95% c.l.

Figure 3.1 (c) Mortality of An.gambiae at intervals over one year after exposure to netting treated with emulsifiable concentrates



—●— Nylon/P 400 —+— Nylon/P 200 —*— Cotton/P 600
 —□— Nylon/L 5 —×— Cotton/L 5

Bars at month 12 = 95% c.i.

	Initial deposit density	mg/m ²	Final deposit density
P400	430		180
P200	210		80
C600	664		425

Table 3.1 (a)

G.C analysis and bioassays of lambdacyhalothrin formulations

G.C analysis = 2 samples (mg/m²)

Bioassay % mortality of 3 replicates (N = 52-57)

95% confidence limits from binomial distribution in parentheses

Fabric/ Formulation	Dilution details	G.C analysis mg/m ²	Bioassay Mortality %
OIL E.C.	Clear liquid with very (2.5%) pungent smell gave a slightly milky coloured emulsion on addition to water		
NYLON	"	6.0; 6.0	98 (90-99.95)
COTTON	"	2.6; 1.4	60 (45-73)
E.W	Thin milky emulsion -(5.0%) gave good emulsion		
NYLON	"	18; 16	98 (91-99.96)
COTTON	"	3.0; 3.0	98 (90-99.96)
C.S (10.0%)	Thick creamy suspension - pale suspension on addition to water		
NYLON	"	16; 13	100 (93-100)
COTTON	"	4.8; 3.2	72 (59-83)
S.C	Grey paste, very viscous (7.5%) gave greyish suspension and slight discolouration of net		
NYLON	"	11; 12	100 (94-100)
COTTON	"	16; 12	63 (47-71)
W.P	Greyish powder-good (10.0%) dispersion on addition to water		
NYLON	"	9.0; 7.0	100 (93-100)
COTTON	"	25; 19	100 (94-100)
Control (netting dipped in water)			2 (0.05-10)

Table 3.1 (b)**Target and observed deposit densities of nylon and cotton netting treated with W.P.s of permethrin and deltamethrin**

Pyrethroid/fibre	Deposit Density (mg/m^2)	
	Target	Observed (2 samples)
Permethrin (25% W.P.) Nylon	200	280; 220
Cotton	800	560; 620
Deltamethrin (2.5%) Nylon	6.0	8.0; 6.0
Cotton	6.0	18; 14

Table 3.1 (c)

Effect of washing on formulations of lambdacyhalothrin

Fibre/Formulation		O Wash	1 Wash	2 Washes
NYLON/E.C.	G.C. ¹	6.0; 6.0	0.85; 0.95	0.35; 0.35
	B.A. ²	100 (95-100)	75 (63-84)	23 (14-35)
COTTON/E.C.	G.C.	5.0; 3.0	3.0; 3.0	2.0; 3.0
	B.A.	76 (64-85)	58 (45-69)	47 (35-60)
NYLON/ OIL E.C.	G.C.	6.5; 7.2	0.75; 0.75	0.4; 0.4
	B.A.	99 (93-99.96)	81 (70-89)	54 (42-66)
NYLON/C.S.	G.C.	16; 13	0.75; 0.75	0.4; 0.4
	B.A.	100 (95-100)	61 (49-72)	52 (39-64)
COTTON/C.S.	G.C.	4.0; 4.0	2.0; 2.3	0.62; 0.55
	B.A.	72 (60-82)	50 (38-62)	41 (30-53)
Control		3 (0.35-10)	4 (.04-8)	0 (0-5)

1 = G.C - 2 samples, mg/m²

2 = B.A - % mortality of 4 replicates (N= 69-74)

95% confidence limits from binomial distribution in parentheses.

Table 3.2

Effect of soap on the retention of permethrin a.i by nylon and cotton fibres.

Washing Treatment		1 Wash	2 Washes
NYLON (350; 320 mg/m²) *			
Cowfat soap, water + rubbing	G.C.	22; 18 ¹	3.0; 3.0
	B.A.	20 ² (10-33)	0 (0-7)
Water + rubbing	G.C.	26; 26	16; 18
	B.A.	14 (6-26)	12 (4-24)
Water only	G.C.	76; 76	53; 45
	B.A.	53 (39-67)	30 (18-45)
COTTON (570; 630 mg/m²) *			
Cowfat soap, water + rubbing	G.C.	640; 520	570; 570
	B.A.	27 (16-41)	16 (8-28)
Water + rubbing	G.C.	580; 530	545; 560
	B.A.	0 (0-7)	8 (2-19)
Water only	G.C.	560; 640	600; 605
	B.A.	12 (0-7)	4 (0.4-13)
Control	B.A.	0 (0-7)	2 (0.05-10)

* Initial deposit density

1 = G.C. analyses - 2 samples, mg/m²

2 = B.A. % mortality of 3 replicates (N= 50-56)

95% confidence limits from binomial distribution in parentheses

Results of second test carried out on cotton netting:
(see footnote above)

COTTON (480; 500 mg/m²) *			
Cowfat soap, water + rubbing	G.C.	485; 492	460; 490
	B.A.	12 (5-24)	6 (1-15)
Water + rubbing	G.C.	440; 456	420; 440
	B.A.	6 (1-16)	9 (8-29)
Water only	G.C.	500; 489	465; 482
	B.A.	12 (5-24)	14 (6-27)

Table 3.3

Effect of soap or detergent wash prior to re-impregnation
on subsequent loading of permethrin

		COTTON mg/m ²	NYLON
One wash- water only	G.C.	590; 590 ¹	54; 68
	B.A.	32 (20-46) ²	40 (26-54)
Re-impregnated	G.C.	1040; 1090	620; 480
	B.A.	60 (46-74)	100 (94-100)
One wash-cowfat soap	G.C.	410; 480	21; 23
	B.A.	20 (10-33)	15 (7-28)
Re-impregnated	G.C.	825; 910	345; 395
	B.A.	52 (37-66)	98 (90-99.95)
One wash-"Foama"	G.C.	435; 495	16; 19
	B.A.	25 (14-39)	12 (5-24)
Re-impregnated	G.C.	895; 930	335; 390
	B.A.	58 (43-71)	94 (85-99)
Unwashed	G.C.	480; 490	410; 435
	B.A.	40 (26-54)	100 (93-100)
Re-impregnated	G.C.	1025; 1070	700; 780
	B.A.	64 (50-77)	100 (94-100)
Control		2 (0.04-9)	0 (0-7)

1 = G.C. analyses of 2 samples mg/m²

2 = Bioassay mortality - 3 replicates (N = 49-56)

95% confidence limits from binomial distribution in parentheses

Table 3.4

Effect of pH 3 and emulsion temperature of 100 °C on uptake and wash resistance of nylon and cotton fibres.

Fibre/Treatment and Target deposit density		0 Wash	1 Wash	2 Washes
NYLON				
E.C. (R.T.*) 400 mg/m ²	G.C.	350; 320 ¹	22; 18	3.0; 3.0
	B.A.	94 ² (84-98)	20 (10-32)	0 (0-6)
E.C. + acid + 100°C 400 mg/m ²	G.C.	490; 390	20; 20	2.0; 2.0
	B.A.	100 (94-100)	8 (2-19)	0 (0-7)
E.C. + acid + 100°C 100 mg/m ²	G.C.	90; 70	7.0; 5.0	1.0; 3.0
	B.A.	65 (51-78)	3 (0.05-10)	3 (0.05-11)
COTTON				
E.C. (R.T.) 800 mg/m ²	G.C.	570; 630	570; 570	610; 520
	B.A.	39 (26-52)	27 (16-41)	16 (8-28)
E.C. + acid + 100°C 800 mg/m ²	G.C.	1180; 860	730; 780	670; 570
	B.A.	62 (48-75)	26 (15-40)	26 (16-40)
E.C. + acid + 100°C 400 mg/m ²	G.C.	540; 560	430; 530	400; 320
	B.A.	36 (24-50)	5 (1-15)	10 (3-21)
Control	B.A.	0 (0-7)	2 (0.05-11)	2 (0.05-10)

* R.T. = Room temperature

1 = G.C. analyses of two samples, mg/m²

2 = Bioassay % mortality of 3 replicates (N= 50-58)

95% confidence limits from binomial distribution in parentheses

Table 3.5

Effect of time spent in the emulsion by nylon and cotton fibres on pyrethroid uptake and washfastness.

Fibre/Treatment		0 Wash	1 Wash	2 Washes
NYLON				
3 minutes*	G.C.	350; 320 ¹	22; 18	3.0; 3.0
	B.A.	94 (84-99) ²	20 (10-32)	0 (0-6)
10 minutes	G.C.	370; 300	15; 15	23; 18
	B.A.	89 (78-96)	2 (0.4-12)	4 (0.4-12)
1 hour	G.C.	320; 300	10; 10	11; 7
	B.A.	100 (94-100)	0 (0-6)	2 (0.4-9)
12 hours	G.C.	360; 290	23; 21	20; 18
	B.A.	96 (88-99.96)	8 (2-16)	5 (1-15)
COTTON				
3 minutes *	G.C.	570; 630	640; 520	570; 570
	B.A.	39 (27-53)	27 (16-40)	16 (8-29)
10 minutes	G.C.	690; 610	390; 420	390; 330
	B.A.	45 (31-59)	23 (13-36)	5 (1-15)
1 hour	G.C.	610; 510	390; 420	300; 350
	B.A.	38 (25-51)	20 (10-33)	9 (3-20)
12 hours	G.C.	870; 1320	700; 670	260; 310
	B.A.	57 (43-70)	13 (5-24)	18 (9-30)
Control	B.A.	0 (0.05-10)	1 (0.05-10)	2 (0.04-9)

* Usual time in emulsion.

1= G.C analyses of 2 samples, mg/m²

2= Bioassay mortality of 3 replicates (N= 54-60)
95% confidence limits in parentheses.

Table 3.6 (a)

Polymer formulations of permethrin - G.C. and bioassay results of treated nylon

Target deposit densities = 400 mg/m²

Formulation		0 Wash	1 Wash	2 Washes
E.C.	G.C.	340; 310 ¹	7.0; 7.0	3.0; 3.0
	B.A.	93 (83-98) ²	25 (15-37)	10 (4-20)
E.C + 5% polystyrene 100,000 m.w	G.C.	490; 360	170; 140	130; 130
	B.A.	83 (72-81)	70 (58-81)	53 (40-65)
E.C + 200,000 m.w polystyrene	G.C.	n.d.	n.d.	n.d.
	B.A.	90 (81-96) ²	86 (75-93)	20 (12-32)
E.C + 300,000 m.w polystyrene	G.C.	n.d.	n.d.	n.d.
	B.A.	90 (80-96)	88 (78-95)	26 (16-38)
E.C + 10% polystyrene	G.C.	610; 550	410; 370	290; 230
	B.A.	64 (52-76)	77 (65-86)	36 (25-48)
E.C + 5% latex [#]	G.C.	420; 470	330; 420	300; 370
	B.A.	0 (0-5)	10 (4-20)	3 (0.4-10)
E.C + 5% Shellac	G.C.	n.d.	n.d.	n.d.
	B.A.	84 (74-92)	44 (32-57)	39 (28-52)
E.C + 10% Shellac	G.C.	n.d.	n.d.	n.d.
	B.A.	23 (14-35)	43 (31-55)	8 (3-18)
Control	B.A.	2 (0.02-8.2)	0 (0-6)	1 (0.04-8)

1 = G.C. analyses of 2 samples, mg/m²

2 = Bioassay % mortality of 4 replicates (N=65-72)

95% confidence limits in parentheses

n.d. = No data

= Latex R8075/157/2

Table 3.6 (b)

Polymer and additive formulations of lambdacyhalothrin -
G.C. and bioassay results.Target deposit densities = 6.0 mg/m²

Formulation		0 Wash	1 Wash	2 Washes
E.C.	G.C.	7.8; 8.6	0.76; 0.8	0.41; 0.41
	B.A.	98 (90-99.95)	78 (65-88)	23 (13-36)
E.C. + 0.5% latex	G.C.	7.1; 7.9	0.91; 0.91	0.41; 0.41
	B.A.	78 (65-87)	56 (42-69)	27 (16-41)
E.C + 5% latex	G.C.	6.0; 8.0	3.0; 3.6	0.25; 0.25
	B.A.	40 (27-54)	28 (17-42)	6 (1-15)
E.C + 0.5% oil	G.C.	6.1; 4.9	0.71; 0.73	0.24; 0.24
	B.A.	83 (71-91)	76 (63-86)	35 (23-49)
E.C + 5.0% oil	G.C.	7.0; 9.0	0.71; 0.77	0.24; 0.24
	B.A.	100 (94-100)	98 (91-99.96)	91 (81-97)
E.C + 0.5% latex + 0.5% oil	G.C.	6.2; 6.2	0.55; 0.55	0.2; 0.2
	B.A.	87 (75-95)	50 (36-64)	20 (11-34)
E.C. + 1% B44	B.A.	85 (73-94)	60 (46-73)	8 (3-19)
E.C. + 1% B67	B.A.	90 (79-96)	57 (43-71)	30 (19-44)
E.C. + 1% B82	B.A.	88 (76-95)	44 (30-58)	18 (9-30)
E.C. + 2.5% B44	B.A.	85 (73-94)	31 (19-45)	10 (4-21)
E.C. + 2.5% B67	B.A.	71 (58-83)	69 (55-81)	17 (8-29)
E.C. + 2.5% B82	B.A.	80 (67-90)	50 (36-64)	4 (0.4-12)
E.C. + 0.5% P.S.*	B.A.	83 (71-91)	60 (46-72)	12 (6-24)
E.C. + 1.0% P.S.	B.A.	89 (75-96)	30 (18-43)	5 (1-15)
E.C. + 2.5% P.S.	B.A.	87 (75-95)	49 (36-63)	3 (0.4-12)
E.C. + 5.0% P.S.	B.A.	92 (82-98)	74 (60-84)	28 (17-42)
Control	B.A.	2 (0.05-10)	4 (0.4-12)	0 (0-6)

1 = G.C. analyses of 2 samples, mg/m²

2 = Bioassay mortality 5 of 3 replicates (N = 54-59)

(5% confidence limits from binomial distribution in parentheses)

* P.S. = Polystyrene (m.w. 100,000)

Table 3.6 (c)

**Permethrin plus latex formulations - bioassay mortality of
An. gambiae**

Latex	0 Wash	1 Wash	2 Washes
R8075/157/1 ¹	0 (0-6) [#]	9 (3-19)	13 (5-24)
R8075/157/2	0 (0-6)	10 (4-21)	3 (0.4-12)
R8075/157/3	2 (0.05-10)	0 (0-7)	7 (2-18)
R8075/157/1 ²	4 (0.5-8)	0 (0-6)	3 (0.4-12)
R8075/157/2	2 (0.04-9)	2 (0.04-9)	0 (0-6)
R8075/157/3	0 (0-6)	7 (2-17)	7 (2-17)
Vinacryl 7172 ¹	7 (2-18)	26 (16-40)	0 (0-6)
Vinamul 18134	14 (6-26)	15 (7-27)	40 (28-54)
Vinamul 18207	5 (1-15)	13 (5-24)	14 (6-26)
Vinacryl 7172 ²	4 (0.4-12)	5 (1-15)	5 (1-15)
Vinamul 18134	0 (0-7)	10 (4-21)	4 (0.4-12)
Vinamul 18207	7 (2-18)	5 (1-15)	7 (2-18)
Control	0 (0-6)	2 (0.04-9)	2 (0.04-9)

1 - Treatment (b) (see 3.6)

2 - Treatment (c) (see 3.6)

- Bioassay % mortality of 3 replicates (N = 52-59)

95% confidence limits from binomial distribution in parentheses

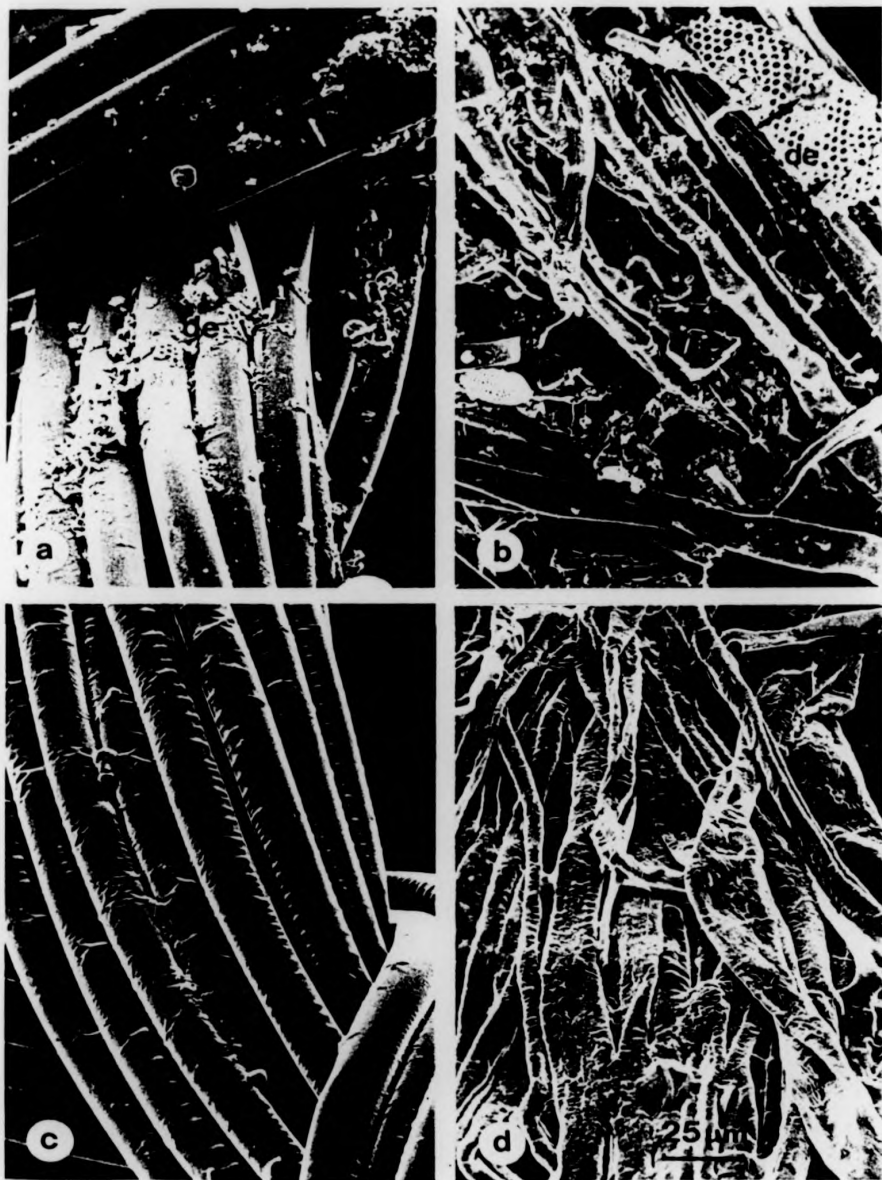
**Plate 3 Scanning electron micrographs of nylon and cotton fibres
treated with wettable powder and wash-resistant formulations
of permethrin**

(a) Nylon treated with 0.8g permethrin/m² W.P
Diatomaceous earth (de) particles visible

(b) Cotton treated with 0.8g permethrin/m² W.P.
**Diatomaceous earth (de) particles visible. Arrows denote active
ingredient adsorbed onto particle.**

**(c) Nylon treated with 0.8g permethrin/m² wash-resistant
formulation. Surface deposit visible.**

**(d) Cotton treated with 0.8g permethrin/m² wash-resistant
formulation. Surface deposit visible.**



CHAPTER 4

NON LETHAL AND VAPOUR EFFECTS OF INSECTICIDE TREATED NETTING

4.1 Rates of knock-down and recovery of mosquitoes exposed to a range of insecticide deposit densities on nylon netting

4.1.1 INTRODUCTION

The studies reported so far have all been concerned with the lethal effects of insecticide treated netting. Conventional bioassays, which only record mortality 24 hours after exposure, do not necessarily give a complete picture of the affects of an insecticide. Such studies give very little indication of (a) sub-lethal uptake or (b) 'overkill'. The latter occurs when mosquitoes receive more than enough insecticide to kill them, which may take place, for example, if knock-down does not happen quickly. Sub-lethally intoxicated insects may not be able to find their normal resting sites or a food source and therefore may be eaten by predators or starve to death.

Knock-down (k.d.) is defined as the state of intoxication and partial paralysis after insecticide exposure, which usually is followed by death (Wickham et al., 1974). It is clearly evident when using pyrethroids which can be divided into two groups, depending on how rapidly they cause knock-down (see section 1.8).

Under natural conditions, insects knocked-down may be trampled under foot or eaten by ants or other predators. The rate of recovery from knock-down will be a crucial factor in determining whether a mosquito recovers from a sublethal dose of insecticide.

The rate at which k.d., subsequent recovery and eventual mortality occurred with mosquitoes exposed to three insecticides was studied in the following experiments.

4.1.2 MATERIALS AND MATERIALS

Nylon netting was treated (Chapter 2) with the E.C.s at the following deposit densities;

permethrin - 800, 400, 200, 100, 50 and 25 mg/m²,

lambdacyhalothrin - 25, 12, 6, 3, 1.5 and 0.5 mg/m²,

pirimiphos-methyl - 400, 200, 100 and 50 mg/m².

In addition, nylon netting was treated at 400 mg/m² of the wash-resistant permethrin formulation (section 3.6) was tested. Rate of recovery of mosquitoes after exposure to unwashed netting and netting washed once or twice was compared with the rate of recovery of mosquitoes after exposure to the ordinary formulation of permethrin, unwashed, washed once or twice.

Ten-15 female mosquitoes, glucose-fed aged 3-5 days, were exposed for three minutes and the following two points were studied:

(a) Time taken for knock-down to occur: at the end of the three minute exposure the mosquitoes were blown through to the recovery section of the tube. The number of mosquitoes knocked-down was recorded every five minutes up to and including one hour post exposure. Mosquitoes were classified as knocked-down if they were unable to support themselves on their legs or fly, even when blown gently.

(b) Recovery from k.d. : this was studied for the permethrin and lambdacyhalothrin treated netting only. After a 3 minute exposure to treated netting, the number of mosquitoes remaining knocked-down was scored every hour for 6 hours. After a 12 hour break during which no scoring took place, the number knocked down was again scored every hour up to 24 hours post-exposure. At that time, those still down were considered to be dead and the proportion that those represented was taken as the mortality.

Four replicates were carried out over a number of days.

4.1.3 RESULTS

(a) Figure 4.1 (a) shows the percentage of mosquitoes knocked down, assessed every five minutes up to an hour after exposure to permethrin and pirimiphos-methyl treated netting. After exposure to the higher deposit densities of permethrin, all the mosquitoes were knocked down 20 minutes after the end of exposure. Percentage knock-down of mosquitoes post exposure to the pirimiphos-methyl treated netting was very low, reaching a maximum after one hour of about 25% with the 400 mg/m^2 deposit. The mosquitoes exposed to the lambdacyhalothrin treated netting usually took longer to succumb to k.d., with only the 2 highest deposit densities causing 100% k.d within the hour (Figure 4.1 (b)). However, a direct comparison of the same deposit density, 25 mg/m^2 , of lambdacyhalothrin or permethrin, shows that the lambdacyhalothrin caused the fastest knock-down.

(b) Figure 4.1 (c) shows the recovery of mosquitoes exposed to the range of deposit densities of the ordinary permethrin formulation and washed and unwashed ordinary and wash-resistant formulations. Little recovery occurred in the first 6 hours post-exposure. The majority of recovery occurred between 6 and 18 hours post-exposure. The deposit which had been washed once had high initial k.d. (70%) but after 24 hours, mortality was only 25%.

Figure 4.1 (d), showing recovery of mosquitoes after contact with the lambdacyhalothrin treated netting, indicates that recovery does not occur as it does after contact with permethrin treated netting. The lower deposit densities (1.5 and 0.5 mg/m^2) caused less initial k.d. than subsequent 24 hour mortality, i.e. there was no recovery but considerable delayed mortality. This was also true of the pirimiphos-methyl - hourly k.d. was not assessed but the final mortality rates are shown on Figure 4.1(a).

4.1.4 DISCUSSION

From this study, the differing modes of action of the two pyrethroids can be distinguished. As pointed out in the introduction (1.8), the pyrethroids can be divided into those with early k.d. but comparatively low kill (e.g. permethrin) and slower k.d. but higher kill (e.g. lambdacyhalothrin).

It is difficult to directly compare two pyrethroids whose insecticidal potency differs so much that both 400 mg/m² of permethrin or 3 mg/m² of lambdacyhalothrin cause the same mortality but, if one divides the deposit densities of the two pyrethroids into those causing 100% mortality (as assessed 24 hours after exposure) and those that do not, one finds 800 and 400 mg/m² of permethrin and 25, 12, 6 and 3 of lambdacyhalothrin in the former category. Knock down of all mosquitoes had occurred 20 minutes after exposure with each permethrin treatment, and by 45 minutes with the 25 and 12 mg/m² of lambdacyhalothrin; it did not occur within the hour for the 6 and 3 mg/m² treatments.

Again, a difference in performance is clear when one records recovery during the hours post-exposure. Mosquitoes exposed to the permethrin treated netting showed little recovery in the first 6 hours. For example, the netting treated with the ordinary emulsion which had been washed once, caused a high initial k.d. although 24 hour mortality was low. However, in the field, knocked down insects would probably be taken by ants or termites or squashed under foot or in bedclothes if they are inside nets. In an experiment conducted under natural conditions 85% of the tsetse flies which fell to the ground due to the knock-down effect were removed within 6 hours and almost all of them disappeared within 12-24 hours (Laveissière *et al.*, 1985). Approximately 10% of mosquito corpses left in the experimental huts overnight to check that the huts were scavenger-proof were removed by the morning (section 6.3).

With the high deposit densities of lambdacyhalothrin (25, 12, 6 and 3 mg/m²) there was high initial k.d. but no subsequent recovery. With the lower deposit

Figure 4.1 (a) Percentage knock-down of *Angambiae* after exposure to netting treated with permethrin or pirimiphos-methyl

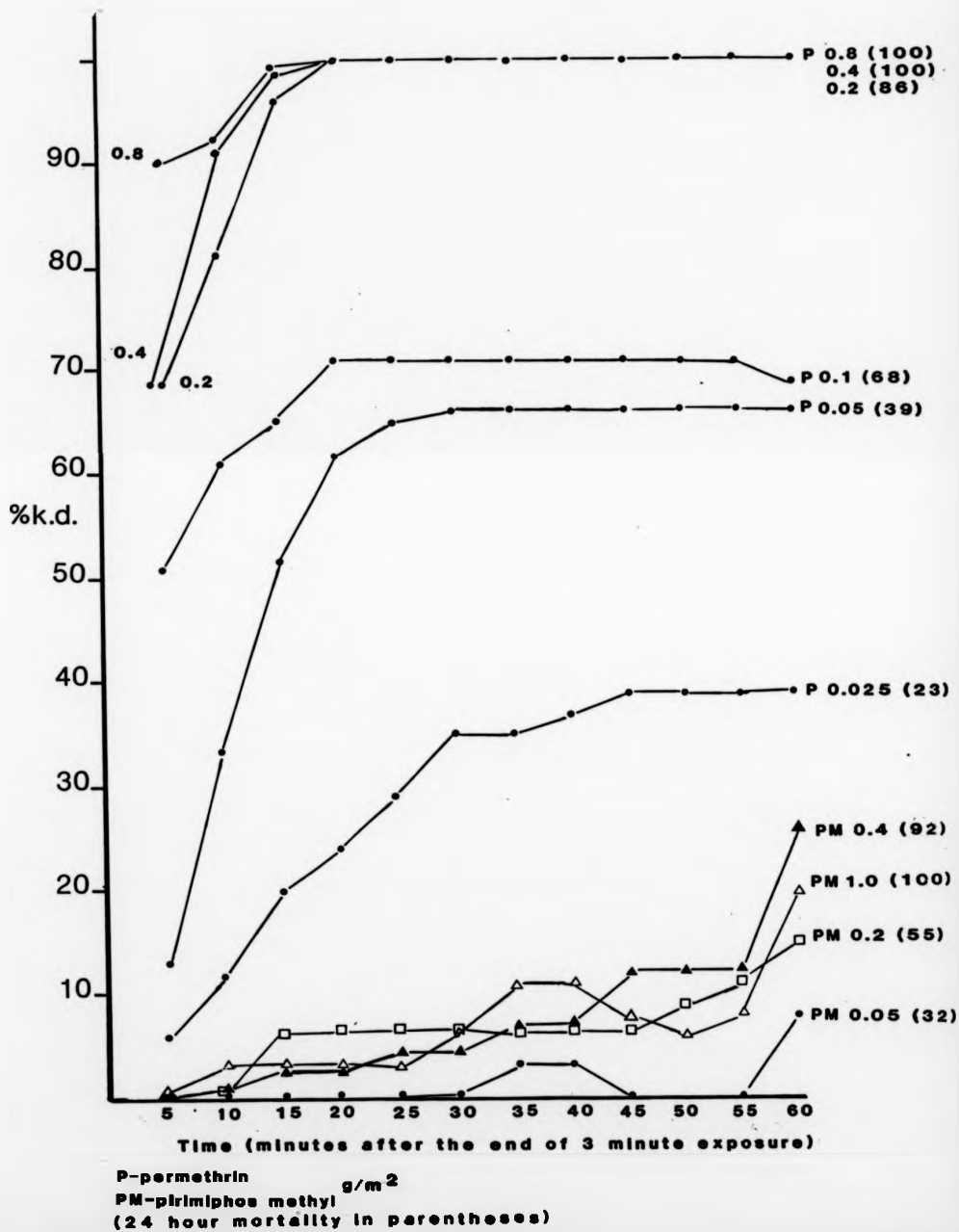


Figure 4.1 (b) Percentage knock-down of *An.gambiae* after exposure to netting treated with lambdacyhalothrin

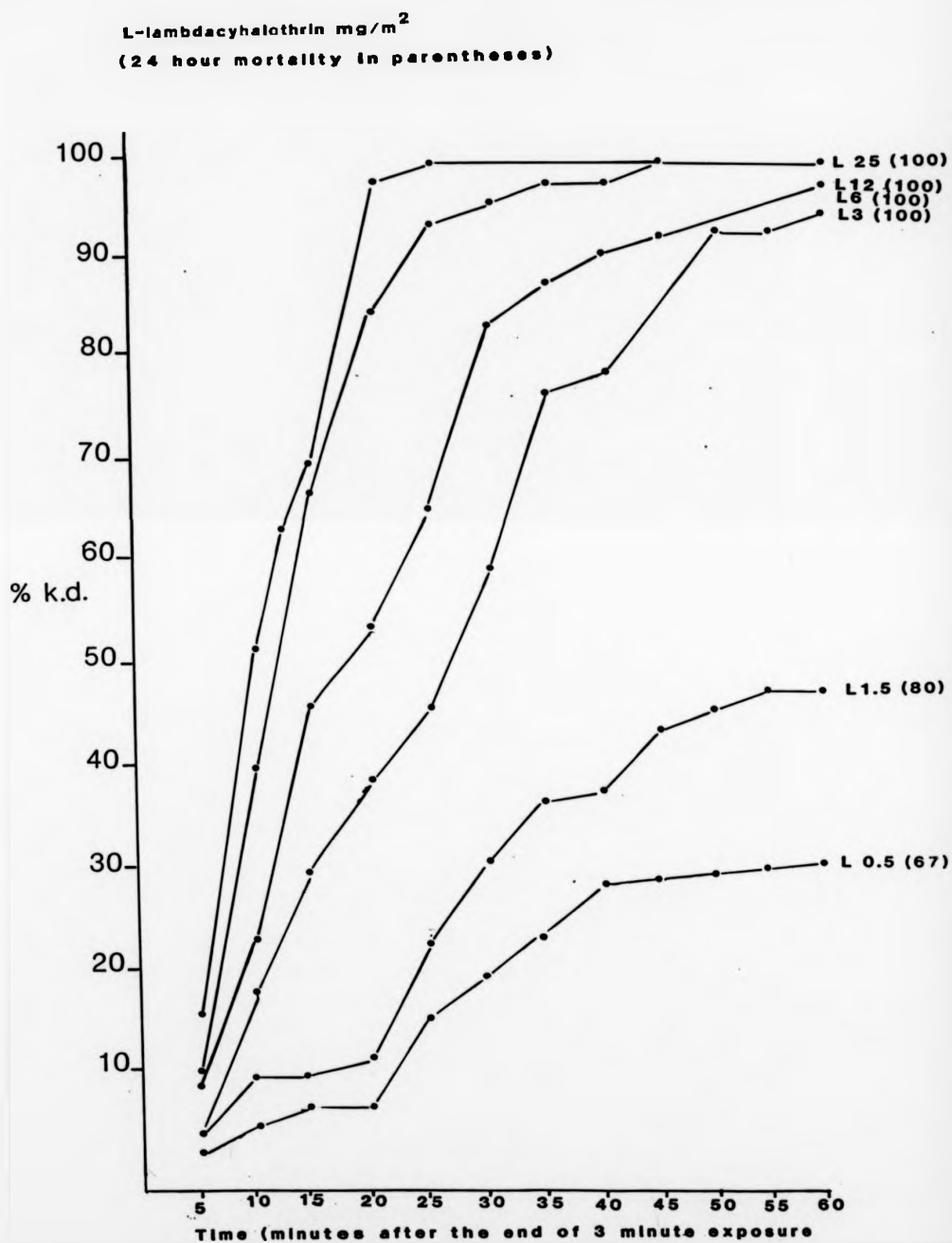


Figure 4.1 (c) Knock-down during the period 1-23 hours and 24 hour mortalities of Angambiae after exposure to permethrin treated netting

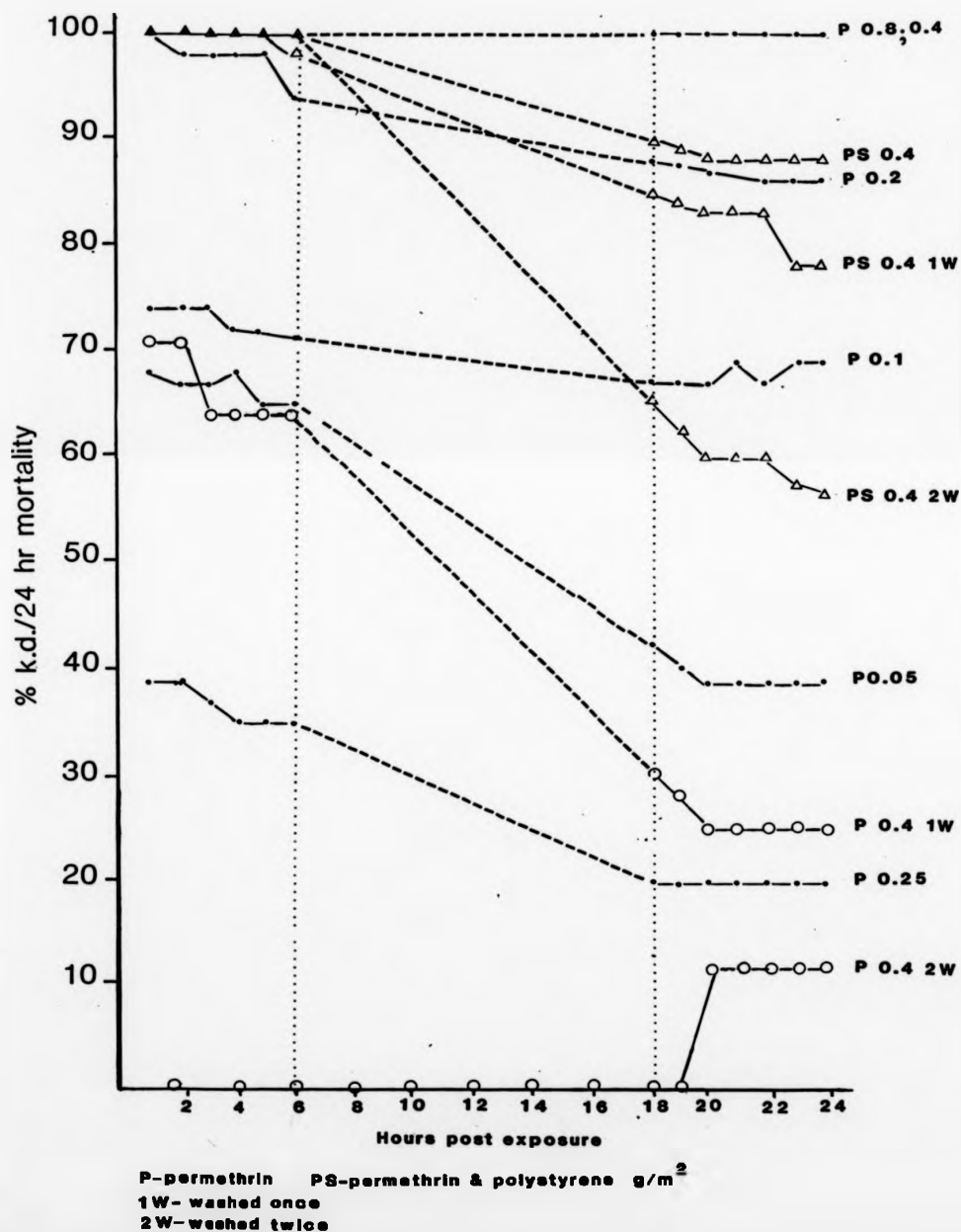
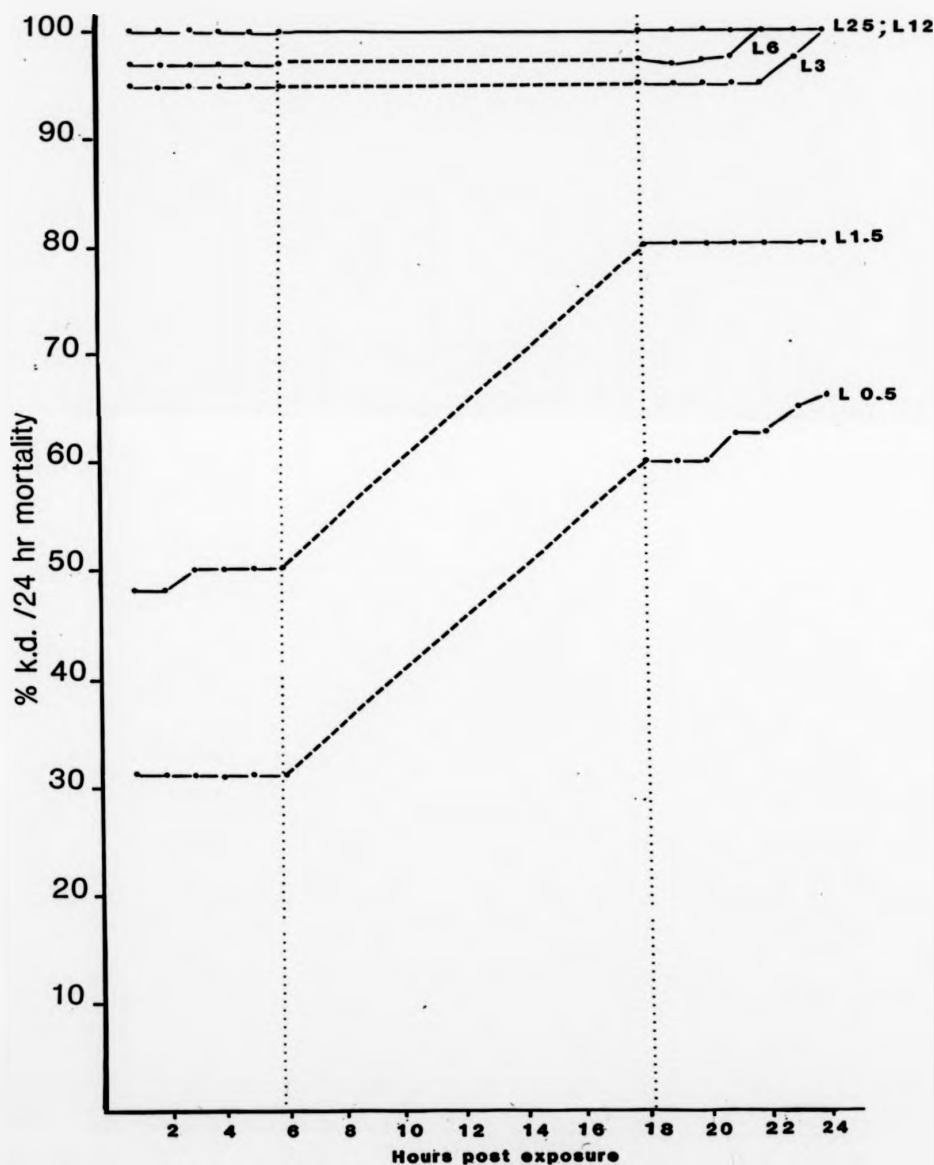


Figure 4.1 (d) Knock-down during the period 1-23 hours and 24 hour mortalities of An.gambiae after exposure to lambdacyhalothrin treated netting

L-lambdacyhalothrin mg/m²



densities, many more mosquitoes died 6 to 18 hours following exposure. Thus, in the field where deposit densities of this pyrethroid had decayed, the mosquitoes might still obtain a blood-meal, thus potentially transmitting malaria, before the insecticide has its lethal effect.

4.2 Relationship of insecticide deposit density to the leg fracture of mosquitoes

4.2.1 INTRODUCTION

A side effect of pyrethroids which has received very little attention is the phenomenon of leg fracture (Khoo and Sutherland, 1981). Leg fracture occurs at the trochanterofemural joint, which is similar to a ball and socket type of joint. Induced leg fracture has been observed in both sexes of several mosquito species including Aedes aegypti, Ae. triseriatus, Ae. sollicitans, Anopheles quadrimaculatus and Culex pipiens.

Leg fracture may affect walking, flight, feeding and oviposition. The aim of the following experiment was to see if insecticide deposit density on netting affected the number of legs lost by mosquitoes exposed to treated netting.

4.2.2 MATERIALS AND METHODS

The insecticides and deposit densities were those used in section 4.1, except that the wash-resistant permethrin was not tested. The mosquitoes were exposed to the treated netting in WHO adult resistance test kits. Up to five female mosquitoes aged 3-5 days were used per deposit density and a number of replicates were carried out on different days. Mosquitoes were exposed for 3 minutes to treated netting or to a control treatment of netting dipped in water only, after which they were given 10% glucose on cotton wool pads. After 24 hours any survivors were killed by placing a pad of cotton wool soaked in ethanol over the gauze top of the tube. Each mosquito was examined under a binocular microscope and the number of legs lost by each

individual was recorded. In most cases legs were lost right up to the thorax but sometimes a stump consisting of the coxa and trochanter remained. This was scored as a leg lost. It was postulated that the number of legs lost per mosquito was binomially distributed. χ^2 Goodness of Fit Test carried out on the observed number of legs lost versus the expected number from the binomial theorem indicated no significant deviation of observed from expected. Thus, each leg may be considered to break or not break with equal probability, regardless of what the other legs on the same mosquito do. Hence it was justifiable to pool the data for all the mosquitoes in each treatment group and confidence limits on the percentage of legs lost were fitted from binomial tables.

4.2.3 RESULTS

Figure 4.2 shows the mean number of legs lost per mosquito at the different deposit densities of the three insecticides. All the pyrethroid treated netting, with the exception of the lambda cyhalothrin at a deposit density of 0.5 mg/m^2 , caused greater mosquito leg fracture than the untreated netting. There was no strong evidence that the deposit densities of the pyrethroids affected the number of legs lost by mosquitoes. The number of legs lost after contact with any of the pirimiphos-methyl treated netting was not significantly different from the untreated netting.

4.2.4 DISCUSSION

Koo and Sutherland (1981) found that the incidence of leg fracture was inversely related to the lethality of the treatment. They state that at levels of bioresmethrin causing complete mortality of *Aedes aegypti* (2.5 ng and higher) fracture was less than at partially lethal and sublethal levels. However, no confidence limits are given for the data. In the present studies the deposit densities of the two pyrethroids which caused 100% mortality also caused greater leg fracture than the

lowest deposit densities of the same compound (with the exception of 400 mg/m² permethrin).

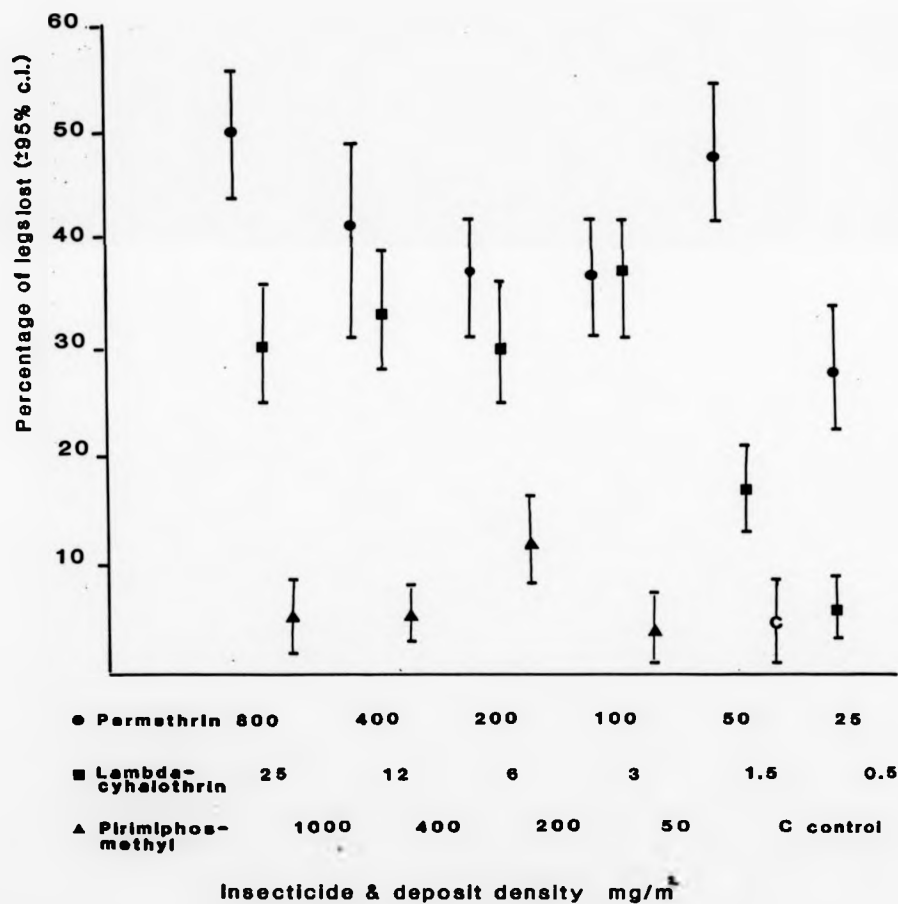
Koo and Sutherland delivered bioresmethrin with a microapplicator and it may be assumed that this leads to a more uniform dosage than in a test kit.

The majority of mosquitoes exposed to the pyrethroid treated netting lost about 2 legs. Leg fracture of the first two legs would be expected to be particularly important in its effect on feeding and that of the last two on oviposition. But, N.Hill (pers. comm.), found that removing either one or two back legs did not have an effect on the number of eggs laid by Anopheles gambiae. However, M.Pile (pers. comm.), has found that the two hind legs of Culex quinquefasciatus are extremely important in the process of oviposition because they are used to shape the egg rafts.

There did not seem to be a pattern in the order of legs lost. Fracture occurred fairly quickly post treatment and appears to be a consequence of strained leg flexion, because treated mosquitoes without leg substrate contact also loose their legs (Khoo and Sutherland, 1981). Clements and May (1977) found with locusts that in addition to acting on neurones, many pyrethroids caused sustained muscle contraction, without any clearly associated nervous activity; the authors suggest that the compounds could have been acting directly on the muscle itself. They also found that many pyrethroids blocked neurally evoked muscle contractions, a result which also may be consistent with a direct effect on the muscle.

The organophosphate, pirimiphos-methyl, caused significantly fewer legs to be lost than either of the pyrethroids and the number lost was not significantly more than that due to the untreated netting. The organophosphates work by inhibiting acetylcholinesterase, so that acetylcholine accumulates at the nerve synapse and proper nerve function is impaired. Excess acetylcholine has widespread harmful effects including uncontrolled hormone release and water loss from the insect integument. The convulsions and hyperactivity observed with the pyrethroid treated netting were not recorded with this organophosphate which probably accounts for the insignificant leg loss.

Figure 4.2 Percentage of legs lost by *An.gambiae* after contact with insecticide treated netting



Blood engorgement by Aedes aegypti was inhibited after treatment with 3 pyrethroids, d-phenothrin, d-allethrin and tetramethrin. The engorgement ratios were 0.5, 0.4 and 0.57 respectively relative to the controls (Liu et al, 1986). They also noticed that the treated mosquitoes lost one or more of their legs after treatment and this, along with other effects of the pyrethroids, may have caused the depression of blood-feeding.

Levinson (cited by Haynes, 1988) suggested the term "insectistatics" be applied to agents that interfere with the normal processes of growth and reproduction, without necessarily leading to mortality. A reduction in oviposition could be due to effects on mate location, courtship, oviposition and associated physiological events such as oogenesis and egg fertilization.

4.3 The effect of different deposit densities of pyrethroids, and organophosphate (OP) and pyrethroid/ OP mixtures on feeding of Anopheles gambiae

4.3.1 INTRODUCTION

The effect of treated netting on blood-feeding by mosquitoes is very important in relation to current developments in malaria vector control. Few laboratory studies have been carried out on this aspect of insecticide treated netting and the only one published to date is by Hossain and Curtis (1989 [b]). They compared nylon and cotton netting of different mesh sizes (1.5, 4.0 and 8.0 mm) at a target deposit density of 200 mg permethrin/m² and concluded that a fresh deposit of 200 mg/m² will prevent mosquitoes from feeding on the limbs of people who come into contact with the treated net in the night. However, it is not known how well decayed deposits of insecticides or deposits left after washing of nets, prevent mosquitoes feeding and/or kill them. As previously mentioned (1.12) fears of pyrethroid resistance have led to

the proposal of treating nets with a mixture of a pyrethroid and an insecticide whose mode of action is unrelated. Thus the following studies using a human test subject were carried out in order to determine:

- (a) the relationship between deposit density on the netting and subsequent feeding, knock-down and mortality of mosquitoes,
- (b) the effectiveness of pyrethroid and organophosphate mixtures as assessed using feeding, knock-down and mortality and the decay of the components of the mixture as assessed using G.C.

4.3.2 MATERIALS AND MATERIALS

Nylon bednets measuring 1m x 1m x 1m (density 31.2 g/m²) were impregnated at the following deposit densities:

Permethrin (25% E.C.) : 50, 200, 400 and 1000 mg/m²,

Lambdacyhalothrin (2.5% E.C.) : 2.5, 6.0 and 25 mg/m²;

Pirimiphos-methyl (50% E.C.) : 50, 200, 400 and 1000 mg/m²;

Permethrin and pirimiphos-methyl (200 and 200 mg/m² respectively);

Lambdacyhalothrin and pirimiphos-methyl (6.0 and 200 mg/m² respectively).

Three to five day old, glucose-fed female An.gambiae were used for the study. Ten to twenty mosquitoes were released for each test and 4 replicates were carried out for each treated net. On every occasion that treated nets were tested a control net (dipped in water only) was also tested.

The tests were carried out in a small room measuring 3m by 2m. The author sat on the floor, under the test net, with one arm pressed firmly against the net so that mosquitoes could easily feed on it through the untreated net. After 30 minutes, the mosquitoes were collected using an aspirator and scored by eye as unfed, partially-fed or fully-fed. Knock-down was also recorded at this stage and 1 hour later. The mosquitoes were held in paper cups with cotton wool soaked in 10% glucose solution

and mortality was scored after 24 hours.

Confidence limits from statistical tables of the binomial distribution were attached to the blood feeding data.

After the feeding experiment the nets impregnated with the insecticide mixtures were hung in a room maintained at about 24°C and 60 % r.h. Every month, for 7 months, pieces were removed and the decay of the components of the mixture nets over time was assessed using G.C. analysis.

4.3.3 RESULTS

The percentage of mosquitoes found fed (partially or fully), percentage knocked-down one hour after the end of the 30 minute exposure period and the percentage dead are shown in Figure 4.3 (a), (b), (c) and (d). All the permethrin and lambdacyhalothrin treated nets (Figure 4.3 (a) and (b) respectively) greatly reduced blood-feeding as compared to the untreated net. The two highest deposit densities of permethrin (1000 mg/m² and 400 mg/m²) significantly reduced blood-feeding compared to the lowest deposit density (50 mg/m²). There were no significant differences in the percentages blood fed at the different deposit densities of lambdacyhalothrin. The percentage of mosquitoes feeding through the pirimiphos-methyl nets did not differ significantly between the different deposit densities or from the control net, with the exception of the 1000 mg/m² net which significantly reduced blood feeding when compared to the control net.

The 95% confidence limits on the mortality caused by the treated nets did not overlap with those of the control net.

The nets impregnated with mixtures both caused a reduction in blood-feeding compared to the pirimiphos-methyl on its own, with mortality remaining at 100%.

Table 4.3 shows the decay of the insecticides on the nets impregnated with the mixtures over 7 months. All insecticides had decayed substantially after 7 months. After 3 months the insecticide deposits on both nets showed reductions of 65-75%.

After 7 months however, the deposit densities of the pyrethroids had not shown any further reductions whereas there was only about 5% of the original deposit density of pirimiphos-methyl remaining on each net.

4.3.4 DISCUSSION

The efficacy of bednets in the field is generally assessed by bioassays using the WHO plastic cone or by release of mosquitoes into the net, if it is intact, for 30 minutes (WHO, 1989). A number of G.C analyses (J.Heales, pers. comm.) have shown that the deposit densities of pyrethroids can be quite patchily distributed over bednets (section 6.1). There have been fears that uneven distribution of insecticide on netting may cause variable pick-up, and hence variable mortality and this may help accelerate the process of evolution of pyrethroid resistance by allowing selective survival of some resistant heterozygotes. Patchy distribution of pyrethroid may also cause bioassay tests to give an unreliable indication of the nets toxic effect to mosquitoes, reinforcing the importance of, where practicable, carrying out several bioassays in different places on one net.

It is reassuring, however, that the results of the study reported above indicate that when a mosquito is hungry and searching for a blood meal the presence of pyrethroid on nets substantially reduces blood feeding; even low deposit densities (e.g those due to ageing or washing of nets) will still cause high mortality when mosquitoes are attracted to human subjects underneath the nets. These studies show that 50 mg/m² of permethrin causes 97% mortality whilst allowing approximately 28% of mosquitoes to feed. The lowest deposit tested of lambdacyhalothrin was similarly as effective. The pirimiphos-methyl nets did not reduce blood-feeding (except at the highest deposit density) but nevertheless caused 100% mortality. Thus using pirimiphos-methyl on nets, mosquitoes could pass on malaria or other mosquito-borne diseases by feeding through the nets but could not pick-up gametocytes, microfilariae etc. without also picking up a lethal dose which would eliminate any

chance of their surviving to become infective. The studies with the nets impregnated with mixtures showed that the addition of pyrethroid to the organophosphate, significantly reduced blood-feeding.

The mixtures were tested with a view to their possible use to forestall the emergence of pyrethroid resistance due to widespread use of pyrethroid impregnated bednets. However, the two components of the mixture must have similar decay rates so that adequate deposits of both insecticides remain available for pick-up by the mosquito over time. Table 4.3 and section 6.2 show that the pirimiphos-methyl decays or evaporates far more quickly than the pyrethroids. Thus it would not be a suitable candidate to be used in a mixture, unless re-impregnation was regularly carried out before the organophosphate lost its effectiveness. Incorporation of polystyrene into the organophosphate and pyrethroid emulsion may make the organophosphate less likely to volatilize although the net treated with the wash-resistant formulation of permethrin was not found to be less deterrent than the one treated with ordinary permethrin (6.2).

It should be mentioned that there are reservations about the use of pyrethroid/organophosphate mixtures on toxicological grounds (WHO,1989). These reservations predominantly concern the labelling of a commercial preparation of an E.C. of the two insecticides which has to give consistent medical advice in the case of poisoning. Since pyrethroids are detoxified by several esterases which are susceptible to inhibition by organophosphates and carbamates (Zuzuki and Muzamoto, 1978) it might be difficult to find antidotes to be used in cases of poisoning by mixtures. The problem might be evaded by mixing the two E.C.s on site and allowing impregnation to be carried out by trained people only. Although it is unlikely that a dry net which had been treated with a mixture would cause any toxicological problems to humans, possible side effects of these and alpha-cyano pyrethroid treated nets should be carefully monitored.

However, more research needs to be carried out to find a more appropriate

Figure 4.3 Effect of deposit density of permethrin, lambdacyhalothrin, pirimiphos-methyl and 2 mixtures on feeding, knock-down and mortality of *An.gambiae*

Figure 4.3 (a) Permethrin

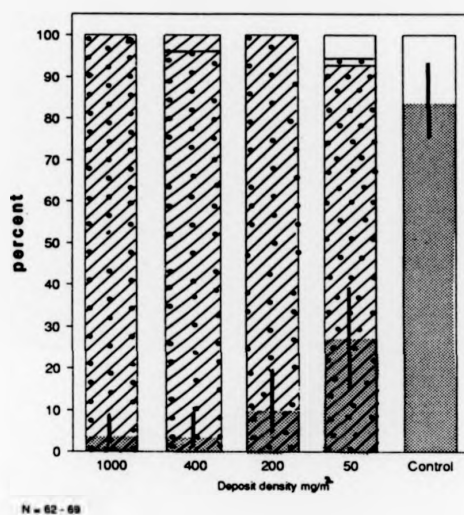


Figure 4.3 (b) Lambdacyhalothrin

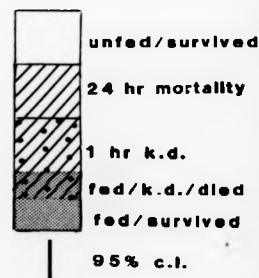
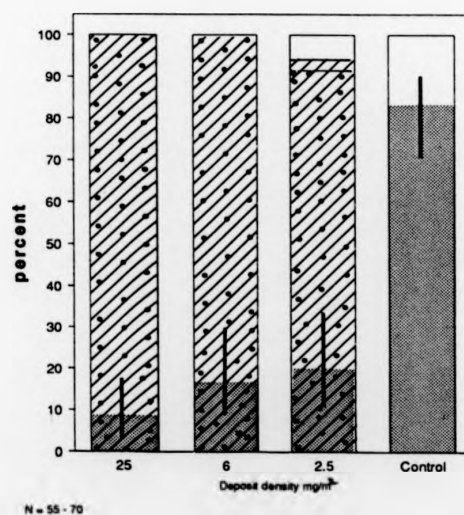
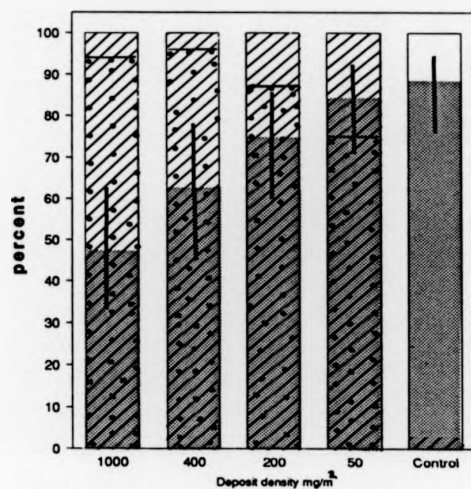


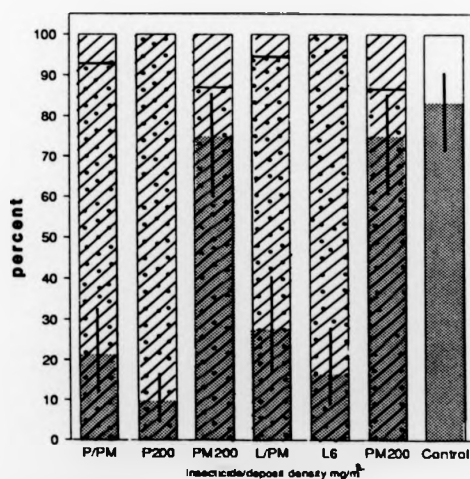
Figure 4.3 Effect of deposit density of permethrin, lambdacyhalothrin, pirimiphos-methyl and 2 mixtures on feeding, knock-down and mortality of *An.gambiae*

Figure 4.3 (c) Pirimiphos-methyl



N = 47 - 57

Figure 4.3 (d) Mixtures and components



P/P/M = 200 200 mg/m N = 47 - 83
L/P/M = 6 200 mg/m

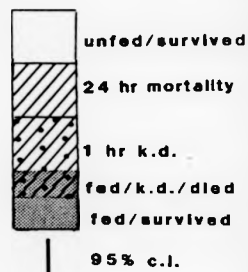


Table 4.3 **Ageing of mixture nets**

2 samples mg/m²

P.M. = pirimiphos-methyl

Months	Permethrin : P.M.		Lambda- cyhalothrin : P.M.	
	200mg/m ²	200mg/m ²	5mg/m ²	200mg/m ²
0	180 ; 155	: 160 ; 120	3.6 ; 4.1	: 151 ; 160
1	85 ; 96	: 61 ; 45	3.2 ; 2.6	: 110 ; 130
2	67 ; 71	: 40 ; 44	1.2 ; 1.4	: 38 ; 36
3	51 ; 53	: 40 ; 35	1.2 ; 1.0	: 39 ; 34
4	61 ; 50	: 24 ; 26	1.4 ; 1.0	: 39 ; 31
5	50 ; 52	: 26 ; 26	1.0 ; 1.0	: 15 ; 14
6	43 ; 44	: 9 ; 10	0.9 ; 1.2	: 10 ; 10
7	47 ; 42	: 6 ; 7	1.0 ; 0.9	: 11 ; 10

mixture than the one reported here.

From the studies described above it is still not clear exactly how long it takes for a mosquito to pick up a lethal dose after it comes into contact with the different deposit densities. When sitting under a net, it is not possible to observe the behaviour of all the mosquitoes released or indeed to keep track of an individual in a room of the dimensions mentioned. A wind tunnel and video recording techniques were therefore used to assess a number of important behavioural aspects due to insecticide treated netting (Chapter 5).

4.4 Vapour toxicity of three synthetic pyrethroids and an organophosphate

4.4.1 INTRODUCTION

When considering the performance of insecticides, a factor frequently considered is the toxic property of the vapour from the insecticide deposits. To assess this factor biologically, both in the laboratory and the field, the commonest practice is to hold test insects in cages a short distance from the treated surface. The toxic effect found after such an exposure is usually referred to as the 'vapour toxicity' (Gerolt, 1959).

It is generally considered that the pyrethroids show little or no vapour activity due to their low vapour pressures, e.g. permethrin = 1.0×10^{-8} mm Hg at 20°C. The organophosphates have high vapour pressures, e.g. pirimiphos-methyl = 1.5×10^{-5} mm Hg at 20°C.

Reports about the vapour toxicity of pyrethroids are conflicting. Ree (1986) reports mortality rates for Anopheles farauti of 100% after being kept for 12 hours 30-50 cm from a net treated with 200 mg permethrin/m². E.Nicholson (pers. comm.) obtained 63% mortality of An.gambiae s.s. after 24 hours exposure to nylon net treated at 200 mg/m². Cyhalothrin (mixture of isomers of lambda-cyhalothrin) caused 25% mortality in mosquitoes maintained 4 mm above filter paper for 16 hours treated at 1000 mg/m² (G.Capel-Williams, unpubl.).

However, M.I.Hossain (pers. comm.) found a bednet treated with 200 mg permethrin/m² had very little vapour effect on mosquitoes. Similarly, Das *et al* (1987) reported that cyfluthrin did not show any appreciable vapour toxicity against Culex quinquefasciatus even at 1000 mg/m².

The aims of the following studies were to:

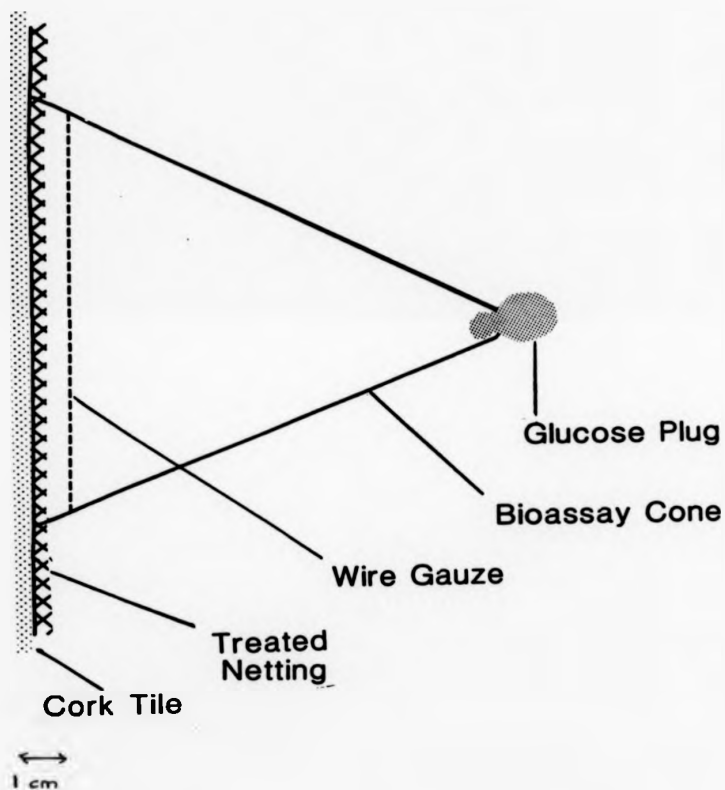
- (a) compare possible vapour toxicity of the three pyrethroids permethrin, deltamethrin and lambdacyhalothrin on treated netting using a closed laboratory system,
- (b) investigate the effect of the E.C.s of permethrin and lambdacyhalothrin undiluted with water, and their blank E.C.s (i.e. those containing no a.i.). The latter were tested to establish if other parts of the formulation besides the a.i. affected mosquitoes (laboratory system).
- (c) assess the possible relationship on vapour toxicity of deposit density of permethrin, lambdacyhalothrin and pirimiphos-methyl on nets in a field situation,
- (d) analyse dust collected in rooms in Tanzania containing treated nets to investigate whether "vapour" mortality may be due to pyrethroid contamination of dust.

4.4.2 METHODS AND MATERIALS

(a) Cotton netting was impregnated with permethrin at 400 mg/m² permethrin (from 25% E.C.),deltamethrin (from 2.5% E.C.) at 5 mg/m² and lambdacyhalothrin at 5 mg/m² (from 2.5% E.C.). The control was netting dipped in water only. The treated netting was attached to a cork tile with drawing pins. A plastic WHO bioassay cone with a piece of wire gauze secured 1 cm from the lip of the cone was attached to the netting (Figure 4.4).

Approximately ten glucose-fed, female mosquitoes were aspirated gently through the hole in the top of the cone which was then plugged with cotton wool

Figure 4.4 Closed testing system for vapour toxicity of pyrethroid impregnated netting



soaked in 10% glucose solution.

Survival (mosquitoes could fly freely), knock-down (mosquitoes were lying on their backs, movement visible but not able to fly) and mortality (no visible movement, even on gentle blowing) were assessed after 24 hours.

Six replicates were carried out over the period of a week.

(b) [i] Ten mls of either E.C. or blank (B.E.C.) of I.C.I. permethrin (25%), Wellcome permethrin (10% E.C.), lambdacyhalothrin (2.5%) or distilled water were pipetted into glass dishes 6 cm deep and 6 cm in diameter. These were each placed in a separate small cage (30x30x30 cm) containing a 10% glucose feeder. Approximately 30 glucose-fed female mosquitoes, aged 3-5 days, were carefully introduced into each cage. Mortality was scored after 2, 16 and 24 hours.

[ii] Since large numbers of mosquitoes were found dead in the dishes containing the formulations, the dishes were covered with a piece of nylon netting which allowed free circulation of vapour but kept the insects a minimum of about 5 cm away from the formulations.

Fresh cages were used for each replicate and two replicates were carried out.

(c) Cages (30x30x30 cm) containing about 30 mosquitoes and a glucose feeder were placed in the centre of 1 m³ nets treated at the following deposit densities:

permethrin - 1000, 400, 200 and 50 mg/m²;

lambdacyhalothrin - 25, 6.0, 2.5 mg/m²;

pirimiphos-methyl - 1000, 400, 200, and 50 mg/m²

Mortality was scored after 12 hours exposure.

(d) Analysis of dust samples. Five samples of dust were obtained from rooms containing treated or untreated nets. The samples were;

A and B = rooms containing nets treated with 30 mg/m² lambdacyhalothrin;

C and D = rooms containing untreated nets, and

E = room containing net treated with 200 mg/m² permethrin.

Extraction procedure:

5 mls of hexane were added to 250 mg of dust sample and the solution was sonicated for 15 minutes. The solution was then blown down to dryness and 2 mls of chloroform added.

Detection used: Mass Selective Detection (M.S.D.- G.C. connected to a mass spectrometer). The M.S.D. can be used in its single ion monitoring mode where it responds to a particular ion. This makes it a very sensitive and selective detector.

4.4.3 RESULTS

(a) Table 4.4 (a) shows the percentages of survival, knock-down and mortality after 24 hours exposure of mosquitoes to the pyrethroid treated netting. Chi-squared tests carried out between pyrethroids to see if there was a significant difference in mortality between any of them found $\chi^2 = 15.5$, d.f = 3, $P < 0.1\%$ for deltamethrin and lambdacyhalothrin.

(b) Both covered (Table 4.4 (b)) and uncovered (Table 4.4 (c)) E.C.s and B.E.C.s caused considerable mortality. Covering the bowls decreased the mortality.

(c) The results of confining mosquitoes in cages under treated nets are shown in Table 4.4 (d). Only the pirimiphos-methyl and lambdacyhalothrin net treated with 25 mg/m² caused significant mortality after 24 hours exposure.

(d) The calculations for the amount of pyrethroid found in the dust samples and the chromatograms from the analysis of sample E and the permethrin standard are shown in Appendix 4.4.

Rooms A and B contained lambdacyhalothrin treated nets, C and D contained untreated nets and E contained a permethrin treated net. The amounts of pyrethroid found in the dust samples were:

A - no lambdacyhalothrin detectable.

Permethrin detected at 2.16×10^{-4} w/w.

B - no lambdacyhalothrin (or permethrin) detectable .

C - Permethrin detected at 3.2×10^{-6} w/w.

D - No pyrethroids detected.

E - Permethrin detected at 1.28×10^{-4} w/w.

4.4.4 DISCUSSION

Both the "open" and "closed" systems of testing gave appreciable mortality after 24 hours. The lambdacyhalothrin treated netting caused the highest observed vapour toxicity, although the rate was not significantly different from that due to a permethrin deposit density 80 times higher than that of the lambdacyhalothrin.

The I.C.I. permethrin E.C. caused higher mosquito mortality than the Wellcome permethrin E.C. in both the uncovered and the covered experiments using the open system. This may have been due to the following:- (i) the I.C.I. formulation contained 25% active ingredient whereas the Wellcome formulation contained 10% active ingredient; (ii) the Wellcome permethrin was 2 years old and may have undergone changes on storage; (iii) the cis: trans isomer ratios of the Wellcome and I.C.I. permethrin are 25:75 and 40:60 respectively and the cis isomer is the more active (toxic) form. With regard to the blank E.C.s, the "lambdacyhalothrin" again showed the highest mortalities. With the uncovered experiments, the Wellcome formulation gave a slightly higher observed mortality after 24 hours (70% compared to 65%) but the difference was not significant by χ^2 test. However, with the covered samples the Wellcome formulation gave a very low 24 hour mortality only 5%, compared to 57% for the I.C.I. formulation. The components of an E.C. are active ingredients, surfactant (emulsifier), solvents, and sometimes oils to thicken the formulation or make it less volatile. The solvents used will have the greatest effect on the vapour toxicity of formulations since they differ in power and volatility. However, one might expect their effect to be relatively short-lived as they would

evaporate as the netting dried.

It is interesting to note the high proportion of mosquitoes found in the uncovered formulations. It appears that they may be overcome by fumes/vapours when flying over the dishes. It seems unlikely that they are attracted to the liquid by thirst, since they are provided with a glucose feeder, and there was no mortality in the control dish which had 10 mls of distilled water in it.

The "closed" system again shows a definite 'vapour toxicity'. However, Gerolt (1959) states that calculation of the concentration of the saturated vapours of many insecticides including DDT and dieldrin make it seem very unlikely that such concentrations are high enough to cause a noticeable mortality, even of susceptible insects exposed for 24 hours. In closed systems, such as the one used in these experiments, the vapours of the pyrethroids may condense on various materials in concentrations sufficient to kill susceptible insects.

It could be argued therefore, that where cases in the literature refer to a "vapour effect" in fact they are referring to surface deposits of sublimed active ingredient in and around insects.

The pirimiphos-methyl treated nets all caused 100% mortality after 24 hours and 3 minute bioassays on a cage which had been left inside, but not touching a net treated at 400 mg/m^2 caused 100 % mortality. This could be very important in the field if nets treated with such an insecticide caused a residual layer of insecticide in the room containing the treated net(s). However, it seems that this organophosphate quickly volatilizes and so would not be suitable for long term use (4.3) unless frequent re-impregnation could be carried out.

However, in an open system, this argument cannot be used because the vapours can disperse without condensation. Another possible explanation of the perceived "vapour effect" of some pyrethroids is that the pyrethroid is carried in dust.

E. Nicholson (unpublished) argues that mortality is not due to permethrin evaporating, because confining mosquitoes in a cage with a bowl of concentrated permethrin (10%), i.e. E.C., did not give significant mortality. She concluded that the

high mortalities obtained (see introduction) may be due to dust becoming impregnated. My results show high mortality with E.C. alone but, considering the relatively low vapour pressures of pyrethroids, the suggestion about dust deserved investigating.

A number of increasingly sophisticated extraction procedures and analytical methods were attempted in the hunt for pyrethroid in dust. Thus it could be concluded that the pyrethroids, if present, were in extremely small quantities. Finally, tiny amounts were found by Mass Selective Detection. Interestingly, there was permethrin detected in samples from rooms with both lambdacyhalothrin and the untreated nets. The occupant of the room containing the lambdacyhalothrin net in which the permethrin was found was actively involved in a treated bed net project and it is quite likely that he could have brought permethrin into the room on his shoes etc. Similarly, although the dust which contained permethrin from the room with the untreated net came from a village using no treated nets, permethrin contamination might similarly have been carried in by the field workers. However, there were 2 samples of dust in which permethrin was not detected, so these explanations are not entirely satisfactory. The extremely small quantities of permethrin should be stressed - it is highly unlikely that a mosquito would come into contact with enough dust for contact with the permethrin contained to be lethal. No lambdacyhalothrin was detected in any of the samples which may be because it was used at much lower deposit densities than permethrin.

The vapour effect is further discussed in section Chapter 6.

Table 4.4 (a)

Survival, knock-down and mortality (%) of An.gambiae being held for 24 hours 1 cm away from pyrethroid treated or un-treated cotton netting.

TREATMENT (mg/m ²)	Survival %	Knockdown %	Mortality %	Number tested
Permethrin (400)	0	13.8	88.16	58
Deltamethrin (5)	3.7	18.5	77.8	54
Lambdacyhalothrin (5)	0	0	100	61
Control (Water)	98.2	0	1.8	56

Table 4.4 (b)

Two, 16 and 24 hour mortalities (%) of An. gambiae in cages containing uncovered E.C.s and B.E.C.s of permethrin and lambdacyhalothrin.

Formulation	IN *	2 Hour OUT **	TOTAL	Mortality (%)			IN	24 Hour OUT	TOTAL
				IN	16 Hour OUT	TOTAL			
PERMETHRIN E.C. (ICI)	0	0	0	55	11	66.0	63	18	81.0
PERMETHRIN B.E.C. (ICI)	2.0	0	2.0	55	8.0	63.0	57.0	8.0	65.0
PERMETHRIN E.C. (WELLCOME)	0	0	0	0	0	0	55	10	65.0
PERMETHRIN B.E.C. (WELLCOME)	2.5	0	2.5	2.5	0	2.5	40	30	70
LAMBDCYHALOTHRIN E.C.	27	5.5	32.5	64.0	20.0	84.0	77.0	23.0	100
LAMBDCYHALOTHRIN B.E.C.	8.0	4.0	12.0	45.0	16.0	61.0	47.0	18.0	65.0
CONTROL (WATER)	0	0	0	0	0	0	0	0	0

* = mosquitoes found in dish containing formulations

** = mosquitoes found outside dish containing formulations

Table 4.4 (c)

Two, 16 and 24 hour mortalities (%) of An.gambiae in cages containing covered E.C.s and B.E.C.s of permethrin and lambdacyhalothrin.

Formulation	MORTALITY %		
	2 Hour	16 Hour	24 Hour
PERMETHRIN E.C. (ICI)	3.7	55.6	70.4
PERMETHRIN B.E.C. (ICI)	0	20.0	56.7
PERMETHRIN E.C. (WELLCOME)	0	0	20.0
PERMETHRIN B.E.C. (WELLCOME)	0	0	5.0
LAMBDA CYHALOTHRIN E.C.	0 0	64.7	76.5
LAMBDA CYHALOTHRIN B.E.C.	0	20.6	32.4
CONTROL (WATER)	0	0	0

Table 4.4 (d)

Mortality of An. gambiae after 24 hours inside, but not touching insecticide treated bednets

Insecticide - mg/m ² - Mortality		
Permethrin	Lambda-cyhalothrin	Pirimiphos-methyl
50 - 7%	25 - 26%	50 - 100%
CTL - 0%	CTL - 5%	CTL - 0%
200 - 0%	6 - 0%	200 - 100%
CTL - 0%	CTL - 2%	CTL - 2%
400 - 0%	2.5 - 4%	400 - 100%
CTL - 4%	CTL - 0%	CTL - 0%
1000 - 4%		1000 - 100%
CTL - 2%		CTL - 2%

CTL = Control

4.5 Acoustic actograph studies on the irritancy of pyrethroid treated nylon and cotton fibres

4.5.1 INTRODUCTION

The control of malaria vectors by residual insecticides (e.g. pyrethroid deposits on bednets) depends on the mosquitoes entering houses and coming into contact with the treated net.

Three kinds of response may impede satisfactory control: (a) natural exophilic or exophagic behaviour patterns, (b) avoidance behaviour because of the presence of insecticides (avoidance, excito-repellency etc (Busvine, 1964)) and (c) if the use of an insecticide selects a strain which differs genetically from the normal in its behaviour, the resulting strain is said to show "behaviouristic avoidance" (WHO, 1960).

Irritability of mosquitoes to several insecticides, notably DDT, is a major problem in the eradication of malaria since it causes the mosquitoes to fly away from treated surfaces before there is sufficient contact for a lethal dose to be picked up.

Species differ naturally in their irritability, some remaining a long time before being excited to fly, whilst others are irritated into flight almost immediately. It must be stressed that the irritant effect of pyrethroids is only an important disadvantage if (a) the insects survive long enough to find another blood meal from someone unprotected by a bednet or (b) it leads to behavioural avoidance by selecting for the refractory behaviour mentioned above.

Mosquito flight involves the emission of a characteristic sound. The acoustic actograph is an electronic system capable of recording flight sound. It was developed by Jones (1964) and Jones *et al* (1967) and responds to the females second harmonic, i.e. 880 Hz. Since the females fundamental (first) frequency coincides with many external noises it is inappropriate to set the triggers to this frequency.

Using this acoustic technique, Jones *et al* (1967) have studied mosquito circadian flight activity. They stated that the circadian flight activity of Anopheles gambiae showed two peaks following light-off and light-on in a LD 12:12 regime.

The actograph normally enables laboratory studies on flight activity to take place over periods of several days without the potentially disrupting presence of a human observer. In the present studies, activity was only monitored for 30 minutes because of knock-down occurring on the pyrethroid treated netting.

The actograph was used to compare the flight activity of female mosquitoes exposed to pyrethroid and blank emulsion impregnated cotton and nylon netting.

4.5.1 METHODS AND MATERIALS

The actograph used in this investigation was similar to that developed by Jones *et al* (1967) and described by Rowlands and Lindsay (1986), and Gornulski (1988). It had 8 chambers housed in a foam rubber insulated wooden box with a glass lid. The membrane floor of each chamber was placed on a rubber ring glued to the face of a microphone. The chambers were packed 10 cm apart, which was sufficient distance to isolate them acoustically, in two layers of foam rubber. The signal made by the mosquitoes' flight sound was fed into an amplifier with a frequency filter to reduce extraneous noise. The output of each amplifier was connected to an electronic trigger which produced an all-or-nothing response to a signal longer than 2 seconds. This resulted in a movement of the marker on a miniscript recorder. The miniscript was set to run at 2 mm per minute.

The chamber consisted of the top half of a standard 250 ml glass reagent bottle (Figure 4.5). The floor and sides of the chamber were covered with cotton or nylon netting impregnated with one of the following:

permethrin (from 25% E.C.): - 400 mg/m²,

deltamethrin (from 2.5% E.C.): - 6 mg/m²,

lambdacyhalothrin (from 2.5% E.C.): - 6 mg/m²,

blank permethrin : equivalent amount of formulation to that needed to give 400 mg/m² (tested on cotton only),

blank lambdacyhalothrin: equivalent amount of formulation to give 6 mg/m^2
(tested on cotton only),
controls: nylon and cotton netting dipped in water only.

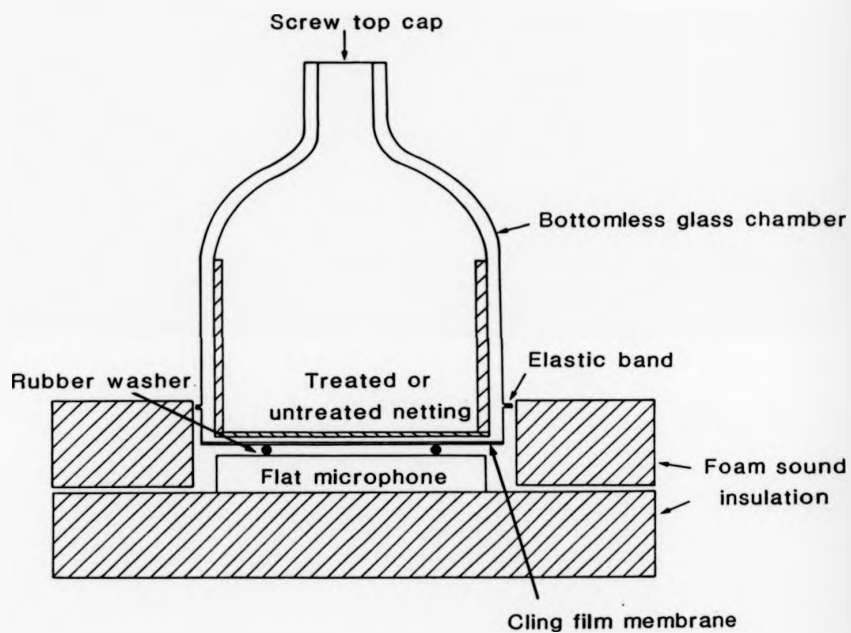
A piece of "cling-film" was stretched tightly across the undersurface of the floor netting to provide a drum-like membrane for transmitting sound into the microphone. The workers previously mentioned, provided glucose and a source of humidity since the mosquitoes' flight activity was being studied for several days. The present studies were carried out for a maximum of 30 minutes and since the glucose and humidity source would have partially covered the netting they were excluded. The temperature in the box was $24 \pm 1^\circ\text{C}$ and the relative humidity was $65\% \pm 5\%$.

The experiments were carried out at "dusk" which was provided by two tungsten bulbs (240 V, 15W) positioned on each side of the box. The light was diffused through a tracing-papered-covered perspex strip to eliminate shadows and point effects.

One 3 day old, glucose fed, female mosquito was gently aspirated into each of the recording chambers and recording started. After 30 minutes the recorder was switched off. Twenty-five to 39 mosquitoes were used per treatment.

The activity trace of each individual on the miniscript roll was quantified by giving it an activity score of 0-10 (inclusive) for each minute (which corresponded to 2 mm on the roll), depending on the number of marks made by the pen. Zero corresponded to no activity (no marks) and 10 to maximum activity.

Figure 4.5 An acoustic actograph chamber (after Gomulski, 1988.)



When all the mosquitoes for each treatment had been scored, the data were entered into a computer program written by Ron Page and Ludvik Gomulski. This calculated the following:

(i) mean activity,

(ii) sliding mean activity (a smoothing function which aids visual analysis of the pattern of activity). The sliding mean A_n for any minute, n , =

$$A_n = \frac{(A_{n-2} + A_{n-1} + A_n + A_{n+1} + A_{n+2})}{5}$$

where A_n is the amount of flight activity in minute n ;

(iii) The total activity (TA) of each mosquito in 30 minutes;

(iv) The time (minutes) by which 50% of the flight activity had occurred for each mosquito (T_{50}).

The total activity and T_{50} values were analysed using the non-parametric Wilcoxon rank-sum test for unpaired data (Sokal and Rohlf, 1980). The statistical analysis of flight activity was of course, based on the data of individual insects and not the sliding mean values.

4.5.3 RESULTS

The flight activity in the presence of cotton, nylon and both cotton and nylon is depicted in histogram form using the sliding means (Figures 4.5 (a),(b), and (c)). The total activity and T_{50} values with 95% confidence limits are shown in Figure 4.5 (d). The results of the rank-sum test are shown in Tables 4.5 (a), (b) and (c).

In all cases the pyrethroid treated cotton netting did not affect the total activity or T_{50} 's of mosquitoes exposed to it. The blank formulations, however, had the following effects:

- (a) the blank "permethrin" treated cotton netting caused the total activity of the mosquitoes to be significantly less than on the control netting (dipped in water only) or on the permethrin treated netting;

- (b) the blank lambdacyhalothrin significantly reduced the total activity when compared to the lambdacyhalothrin treated netting.

The permethrin and lambdacyhalothrin treated nylon netting caused significant differences in mosquito total activity and T_{50} (Table 4.5 (b) and Figure 4.5 (d)). The permethrin treated nylon netting caused a significant difference in the TA and T_{50} of the mosquitoes compared to the control netting, and the lambdacyhalothrin netting significantly affected the mosquitoes total activity.

The comparison of nylon and cotton control netting showed no differences in either TA or T_{50} . But with the pyrethroid treated netting, in all cases the nylon caused very high initial activity of mosquitoes prior to knock-down which occurred in the 30 minutes of exposure in most cases (Figure 4.5 (b)).

The cotton treated netting caused lower activity for a longer period of time with less knock-down. The times by which 50% of the activity had occurred were much shorter on nylon than on cotton, e.g. approximately 6 minutes compared with approximately 15 minutes for permethrin (Figures 4.5 (c) and (d)). There was a significant difference in the total activity between deltamethrin and lambdacyhalothrin on nylon ($p < 0.05$) but no significant difference between their respective T_{50} values. On cotton there was no significant difference between the TA or T_{50} values of lambdacyhalothrin and nylon.

4.5.4 DISCUSSION

A clear difference in the irritancy of the two fibres is shown by these results. The T_{50} corresponds to the irritancy of the pyrethroid/fibre combination. The total activity relates to the knock-down which is indicated on the histograms.

Comparing the total activity of the mosquitoes on the treated cotton and nylon netting does not yield a statistically significant result. However, comparison of T_{50} values gives significant differences at the 0.01 level and below. In all cases T_{50} values were significantly lower on the pyrethroid treated nylon than on cotton netting,

Figure 4.5 (a) (i) Flight activity of An.gambiae after contact with treated or untreated cotton netting

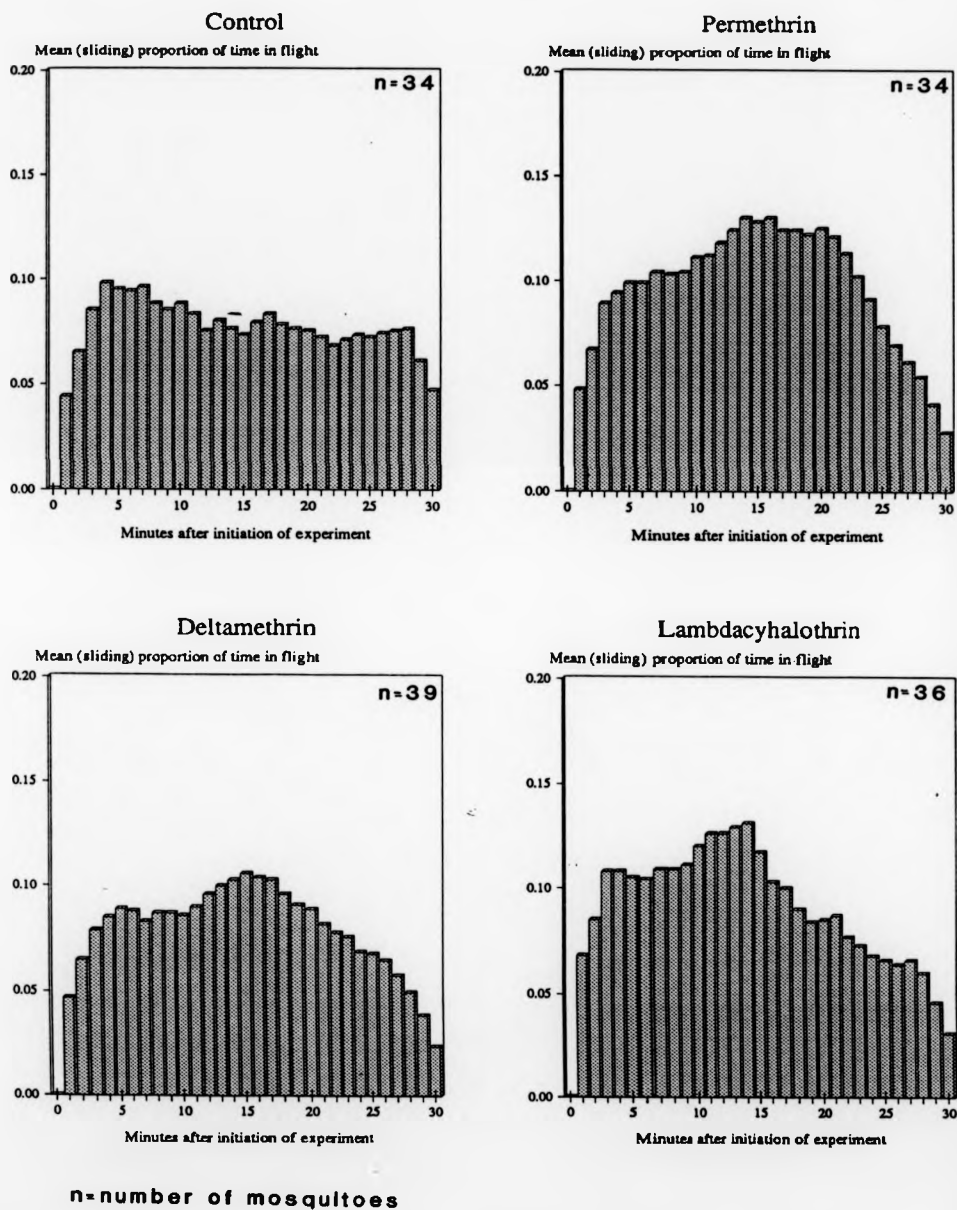
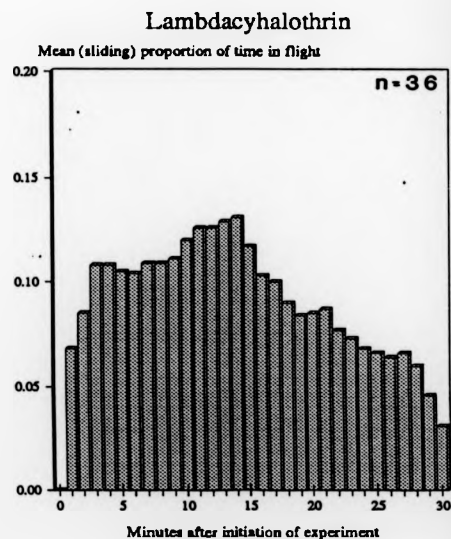
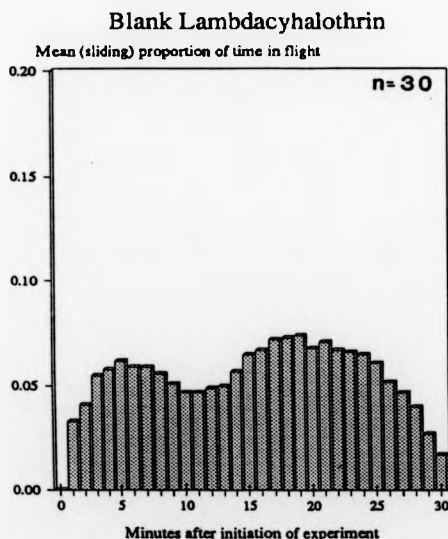
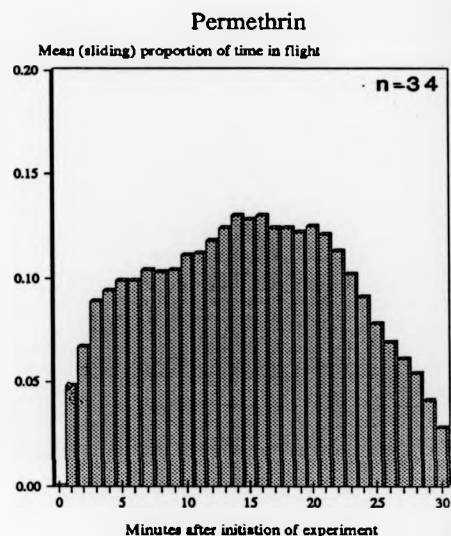
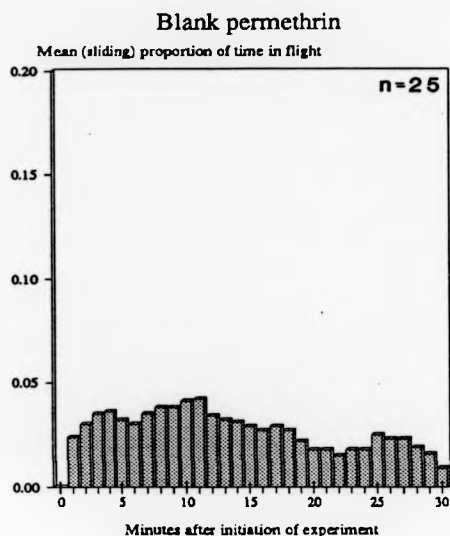


Figure 4.5 (a) (ii) Flight activity of An.gambiae after contact with cotton netting treated with pyrethroid or blank formulation



n=number of mosquitoes

Figure 4.5 (b) Flight activity of An.gambiae
after contact with treated or untreated nylon netting

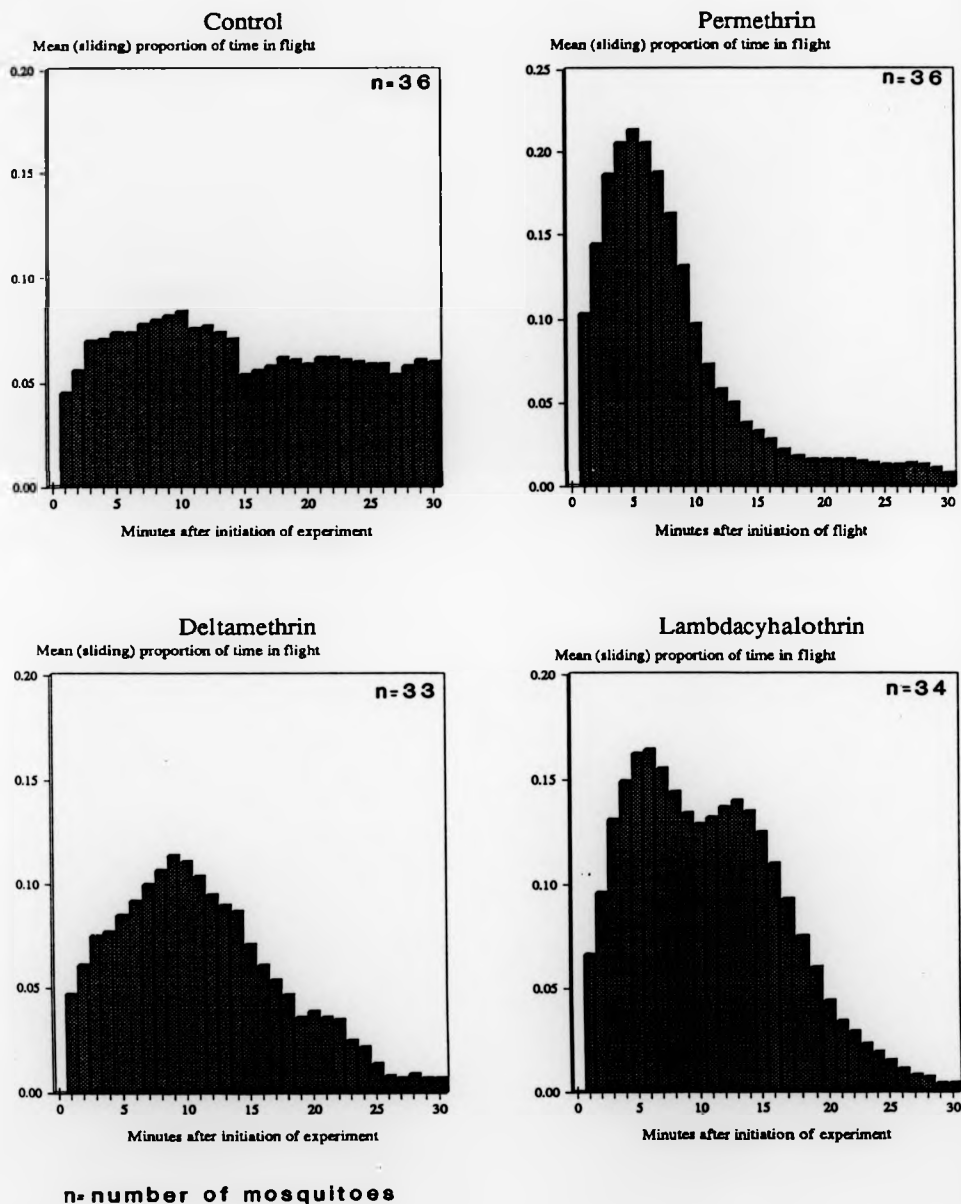
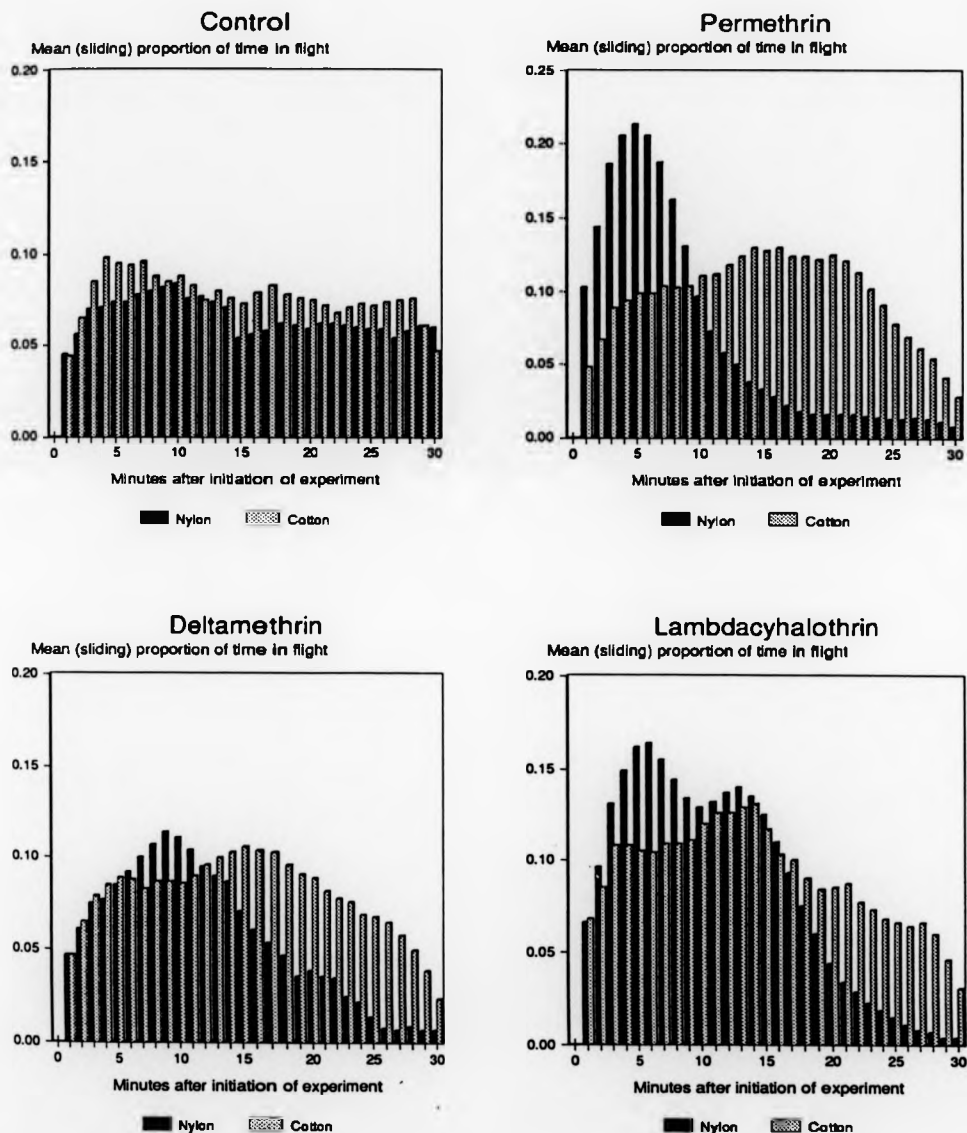


Figure 4.5 (c) Comparison of the flight activity of An.gambiae after contact with treated or untreated cotton and nylon netting



**Figure 4.5 (d) Total activity and time for 50% of the activity (T50)
to occur on treated netting**

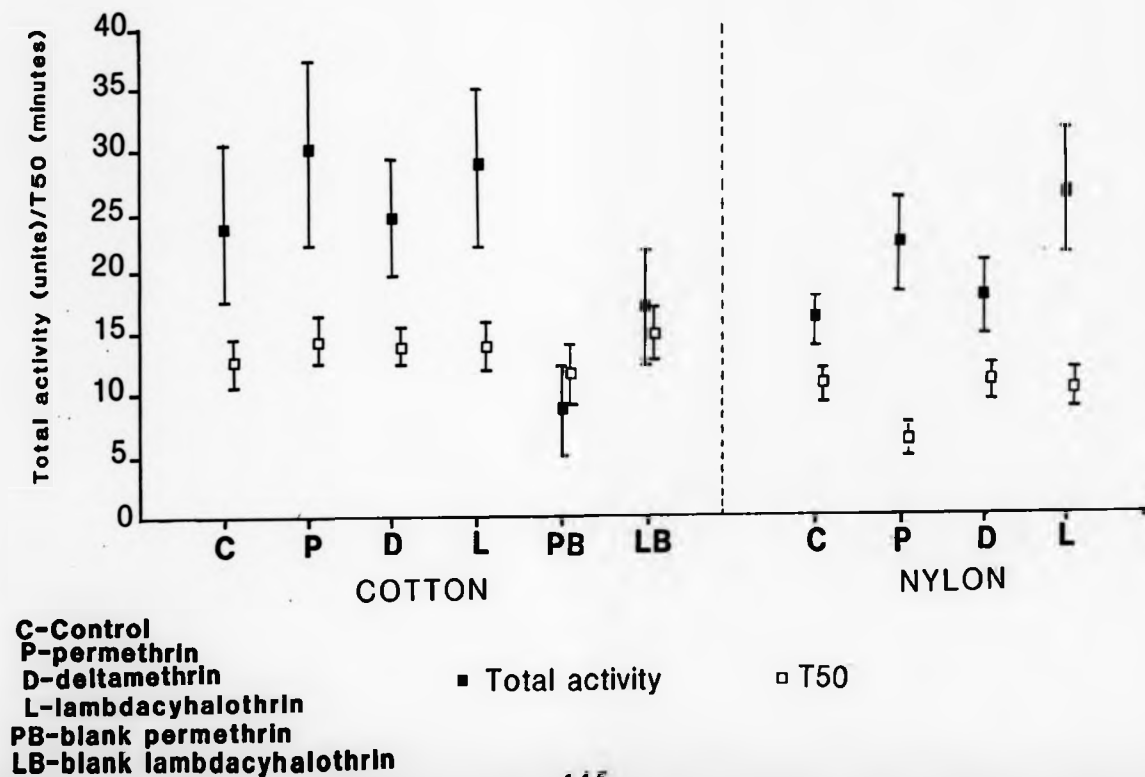


Table 4.5 (a)

Comparison of treated cotton netting using the Wilcoxon Rank-Sum Test.

Comparison of Treatments	Total activity in 30 minutes		Time for 50% of the activity to occur	
	U _s	S.L	U _s	S.L
Treatment v Control				
Permethrin	1.111	N.S	1.254	N.S
Deltamethrin	0.520	N.S	0.775	N.S
L-cyhalothrin	1.105	N.S	0.748	N.S
P Blank	3.709	***	0.650	N.S
L Blank	1.373	N.S	1.429	N.S
Treatment v Permethrin				
L-cyhalothrin	0.241	N.S	0.459	N.S
Deltamethrin	0.863	N.S	0.455	N.S
P Blank	4.723	***	1.822	N.S
Treatment v L-cyhalothrin				
Deltamethrin	0.610	N.S	0.207	N.S
L Blank	2.789	**	0.529	N.S

S.L = Significance level:-

N.S - Not significant

** - Significant at 0.01 level

*** - Significant at 0.001 level

Table 4.5 (b)

Comparison of treated nylon netting using the Wilcoxon Rank-Sum Test.

Comparison of Treatments	Total activity in 30 minutes		Time for 50% of the activity to occur	
	U _s	S.L	U _s	S.L
Treatment v Control				
Permethrin	2.136	*	4.639	***
Deltamethrin	1.605	N.S	0.062	N.S
L-cyhalothrin	3.075	**	0.354	N.S
Treatment v L-cyhalothrin				
Permethrin	1.123	N.S	4.075	***
Deltamethrin	1.996	*	0.396	N.S
Treatment v Deltamethrin				
Permethrin	0.914	N.S	4.432	***

S.L = Significance level:-

N.S - Not significant

* - Significant at 0.05 level

** - Significant at 0.01 level

*** - Significant at 0.001 level

Table 4.5 (c)

Comparison of cotton and nylon treated netting using the Wilcoxon Rank-Sum Test.

		N Y L O N			
		C	P	D	L
C	C TA	1.869 N.S			
	T50	1.789 N.S			
O	P TA		1.499 N.S		
T	T50		5.569 ***		
T	D TA			1.776 N.S	
O	T50			2.704 **	
N	L TA				0.412 N.S
	T50				2.573 +

C = control, P = permethrin, D = deltamethrin and L = Lambdacyhalothrin

TA = Total Activity

T50 = time by which 50% of the activity occurs

- # : Value of U_s where :-
 N.S - Not significant
 * - Significant at 0.05 level
 ** - Significant at 0.01 level
 *** - Significant at 0.001 level
 + - Significant at 0.02 level

i.e. the treated nylon netting was more irritant to the mosquito initiating greater flight activity. This was probably due to faster pick up of the pyrethroids which form surface deposits on nylon (plate 2).

None of the pyrethroid treated cotton fibres caused increased flight activity of mosquitoes. A comparison of the deltamethrin treated nylon netting with the control nylon netting shows no significant difference in the TA or T_{50} . This parallels the bioassay results (Chapter 2) which showed that the deltamethrin did not kill significantly better on one or other of these two fibres.

By contrast, both permethrin and lambda-cyhalothrin killed significantly better on nylon than on cotton and also showed significant differences in T_{50} on nylon compared to the nylon control.

The two blank formulations decreased flight activity (Figure 4.5 (a)). They had a vapour toxic effect (section 4.4) and the blank formulation of permethrin was found to deter mosquitoes from entering huts (Lindsay *et al.* in press and chapter 6). These results underline the importance of testing the behavioural effects of the other ingredients of an insecticide formulation.

The irritant effect of pyrethroids is only important if the mosquitoes survive. Thus the time taken for a mosquito to pick up a lethal dose from pyrethroid treated netting was investigated (section 4.6). This was then related to the more realistic situation of a mosquito searching for a blood meal (Chapter 5).

4.6 Time taken by An.gambiae to pick up a lethal dose from pyrethroid impregnated netting

4.6.1 INTRODUCTION

As discussed in section 4.5, the irritability caused by insecticides is only a problem if it diverts feeding from protected people to unprotected people or causes behavioural resistance. In order to determine the time taken for a mosquito to pick up

a lethal dose, mosquitoes were exposed to various deposit densities of permethrin and lambdacyhalothrin.

4.6.2 METHODS AND MATERIALS

A single, 3-5 day old, glucose-fed, female mosquito was placed in the tip (2 cm long) of a 50 ml polyethylene conical tube (Sarstedt, W.Germany) and was prevented from escaping with a piece of card over the open end. This was placed on the piece of treated netting, standing vertically in a cardboard frame. The netting treatments were permethrin at 50, 400 and 1000 mg/m² and lambdacyhalothrin at 2.5, 6.0 and 25 mg/m².

The mosquito was exposed to the netting by drawing back the cardboard. A hand was placed behind the netting in order to encourage the mosquito to probe and remain in contact with the netting. A stop watch was started as soon as the mosquito came into contact with the netting, stopped when contact ceased and restarted when contact was renewed.

Twenty-30 mosquitoes were exposed to each time in a range of times from 1 to 480 seconds. After exposure, mosquitoes were placed in paper cups with a pad of cotton wool soaked in glucose on the netting cover. Mortality was scored after 24 hours. Exposure times were log transformed and the data of number killed out of number tested was analysed using the program referred to in Chapter 2.

4.6.3 RESULTS

The results are shown in Table 4.6. The LT₅₀ values ranged from approximately 243 seconds at 50 mg permethrin/m² to 23 seconds at 1000 mg permethrin/m². On lambdacyhalothrin treated netting, the LT₅₀ values ranged from 18 seconds on 2.5 mg/m² to 3 seconds on 25 mg/m².

4.6.4 DISCUSSION

With the permethrin treated netting there does not seem to be a constant relationship between deposit density and time taken to pick-up a lethal dose. For example, the LT_{50} values associated with the netting treated at 50 and 1000 mg/m^2 are 243 and 23 seconds respectively. If the time taken to pick-up a lethal dose was directly proportional to deposit density, one would expect 20 fold differences in the times. Hossain and Curtis (1989 [a]) found, using bioassay kits for exposure which is a much less accurate method than that reported here (particularly at lower deposit densities), that permethrin was disproportionately effective at shorter exposure times. They speculated that this was because permethrin does not penetrate into mosquitoes at a constant rate but does so faster during initial exposure before part of the penetration mechanism is saturated. One might also reason that with the longer exposure periods mosquitoes are becoming irritated so that they are not remaining in contact with the netting and are therefore less exposed to the insecticide. However, in the present study, immediately a mosquito left the netting, timing was stopped, therefore only total contact time was measured, i.e. the results are an accurate reflection of insecticide exposure.

Although it appears that pick-up of permethrin may be faster at shorter exposure times, this result should not be extrapolated to all pyrethroids. The results from the lambdacyhalothrin treated netting indicate that there is a simple inverse relationship between deposit density and time taken to pick-up a lethal dose, e.g. the LT_{99} value of the 2.5 mg/m^2 is about 10 times more than that of 25 mg/m^2 . Most other insecticide groups show a constant relationship between dose and exposure time, i.e. doubling the dose and halving the exposure time is a constant.

The results indicate that the insects need to be in contact with netting treated with lambdacyhalothrin at 25 mg/m^2 for about 21 seconds to pick-up a lethal dose. However, the artificiality of the situation should be stressed. The mosquitoes are kept in close contact with the treated netting at all times and may be affected by vapours.

Video studies (Chapter 5) were therefore undertaken to compare the lethal times thus obtained, with the times that a hungry mosquito spends in contact with treated netting, when searching for a blood meal.

Table 4.6

Lethal times on Permethrin and Lambdacyhalothrin
impregnated nylon netting.

Pyrethroid mg/m ²	LT ₅₀ (±95% c.l.)	LT ₉₀	LT ₉₅	LT ₉₉
Permethrin				
50	243 (206-282)	395	453	587
400	52 (38-71)	176	247	471
1000	23 (16-31)	91	135	282
Lambda- cyhalothrin				
2.5	18 (10-27)	67	97	194
6.0	19 (12-26)	46	60	98
25.0	3 (5-6)	9	12	21

All times in seconds

4.7 SUMMARY of Chapter 4

- (1) The rates of knock-down and recovery differed between permethrin and lambdacyhalothrin. Generally permethrin treated netting caused fast knock-down and slow recovery of mosquitoes whereas lambdacyhalothrin treated netting was associated with slower knock-down and considerable delayed mortality.
- (2) The deposit density of insecticide on netting did not affect the number of legs lost by mosquitoes. Permethrin and lambdacyhalothrin treated netting caused significantly more legs to be lost than pirimiphos-methyl treated netting which did not cause higher leg loss than untreated netting.
- (3) Nets treated at a range of deposit densities of permethrin and lambdacyhalothrin significantly reduced blood feeding compared to an untreated net. Only the highest deposit density of pirimiphos-methyl (1000 mg/m^2) reduced blood-feeding as compared to the untreated net. Mixtures of pyrethroid and organophosphate (OP) treated nets caused reductions in blood-feeding compared to the OP alone with mortality remaining at 100%.
- (4) Considerable mosquito mortality was found after: (a) confining mosquitoes at least 1 cm away from treated netting for 24 hours, (b) keeping mosquitoes in cages containing bowls of E.C.s and blank E.C.s and (c) holding mosquitoes in cages inside nets treated with a range of concentrations of pirimiphos-methyl or 25 mg/m^2 lambdacyhalothrin. Very small quantities of permethrin (approximately $0.25 \text{ } \mu\text{g/cm}^3$) were found in dust samples taken from rooms containing treated nets and

untreated nets.

- (5) Pyrethroid treated nylon caused higher flight activity of An.gambiae than pyrethroid treated cotton. Cotton netting treated with blank formulations of permethrin and lambdacyhalothrin decreased flight activity compared to their respective formulations and the untreated cotton.
- (6) The time taken for a mosquito to pick up a lethal dose of insecticide from insecticide treated netting was established on permethrin and lambdacyhalothrin treated nylon netting. Examples of LT_{50} values found are approximately 50 seconds on nylon treated at 400 mg/m^2 permethrin and 3 seconds on nylon treated with 25 mg/m^2 lambdacyhalothrin

CHAPTER 5

STUDIES ON INSECTICIDE TREATED NETTING USING VIDEO RECORDING TECHNIQUES

5.1 INTRODUCTION

Wind tunnels have been used to study the mechanism by which many insects locate the source of an attractant, e.g. volatile sex pheromones, host plant and animal windborne odours. They were used by Omer (1979) to study the response of mosquitoes to air currents containing CO_2 and the odour of a human hand.

The aim of the following work was to examine more closely the effect of insecticide on natural behaviour patterns by observing mosquitoes in an odour plume as they came into contact with netting treated with various insecticides while attempting to locate a host animal. Unlike the bioassay method using WHO kits for assessing the toxicity of treated netting (Chapters 2 and 3), a wind tunnel with a live animal bait allows one to test the effect of insecticides on mosquitoes under conditions which are similar to those encountered in the field, i.e. the mosquitoes are simultaneously exposed to host stimuli and the insecticide, and they are free to move away from the insecticide. Unlike the experiments with whole nets (4.3) where a number of mosquitoes were released at once, in the following procedure mosquitoes were released singly so that each individual's behaviour was recorded.

The effects of a number of netting treatments on the mortality, knock-down (k.d.) and irritability of An. gambiae and Culex quinquefasciatus were studied. A deposit derived from ordinary permethrin E.C. was compared with permethrin incorporating polystyrene (wash-resistant formulation) to determine whether the polystyrene reduced the irritancy or toxicity of the permethrin. Low concentrations of permethrin (50mg/m^2) and lambdacyhalothrin (2mg/m^2) were compared with the recommended field doses of 400 and 25mg/m^2 to see if low doses reduced irritancy but still caused significant mortality. A range of three concentrations of each of these

pyrethroids was used to test for a relationship between dose and response. A mixture of permethrin and pirimiphos-methyl was studied to determine whether pirimiphos-methyl masked irritancy or k.d. Cotton netting samples treated with either of two doses of permethrin were compared with nylon. Hossain and Curtis (1989 [a]) found Cx. quinquefasciatus to be less susceptible to permethrin treated nylon than An. gambiae using 2 minute bioassays. In the present study both species were used with the field doses of permethrin and lambdacyhalothrin to further elucidate species differences in response to pyrethroid treated netting.

5.2 METHODS AND MATERIALS

An. gambiae s.s. and Cx. quinquefasciatus were reared to the pupal stage as specified in Appendix 2.2. Approximately 50 pupae of the species to be used were put in pupal bowls and placed in a white netting cage (15cm x 15cm x 15cm) containing a 10% glucose feeder. To maintain a relative humidity of $65\% \pm 5\%$ a pad of damp cotton wool was put over the top of the cage and the cage was placed in a plastic bag. The cage was put in a large wooden box approximately 1m^3 which was lit by a fluorescent strip light set to a 12h:12h light:dark regime. Experiments were conducted 6-8 hours after the light went off since Jones et al (1972) have shown that An. gambiae have a period of maximum activity 6-10 hours after dusk. Five-day-old An. gambiae and seven-day-old Cx. quinquefasciatus sugar-fed females were used. Fourteen to twenty-two mosquitoes were used for each treatment.

5.2.1 The Wind Tunnel

The wind tunnel (Figure 5 (a)) was 110cm long, 40cm wide and 40cm high. It was made from polythene tubing attached to a "handy angle" frame. The tubing was changed between treatments to avoid contamination. A 10 m piece of polythene tubing extended from the downwind end of the wind tunnel to outside the test room to

eliminate exhaust air. The fan (Breeza Fans^R with speed controlled motor, 250 - 1400 r.p.m) was set at 50 on the dial which corresponded to approximately 700 r.p.m. This gave a wind speed of 0.5 m/s through-out the tunnel as measured using a Solomat^R mpm (500 E) anemometer.

Mosquitoes were released into the downwind end of the tunnel from a clean WHO tube lined with filter paper. The temperature and relative humidity were measured inside the wind tunnel on each occasion before starting the experiments, and were found to be $62\% \pm 4\%$ and $24 \pm 2^{\circ}\text{C}$.

5.2.2 Lighting

The wind tunnel was lit by a tungsten filament strip light diffused through a layer of tracing-paper and white opal perspex to eliminate shadows and bright spots. This created a light intensity of about $0.1 - 0.3 \text{ W/m}^2$ as measured using a Young Photometer.

5.2.3 Host

A guinea-pig was kept in a cage upwind of the netting so that the wind tunnel was filled with a turbulent mixture of clean air and host odour as the fan blew across the guinea-pig. The same guinea pig was used throughout the study.

5.2.4 Area surveyed

A video camera was positioned to view the entire area of netting (30 x 24cm) and 15cm downwind of the netting.

5.2.5 Mosquitoes

One mosquito at a time was placed in the release tube and left to become acclimatized for one minute. The door of the tube was then slid open, allowing the mosquito to enter the wind tunnel. Mosquitoes which did not leave the entry tube

within 5 minutes were discarded. The behaviour of each mosquito was recorded for 10 minutes or until knock-down occurred. A mosquito was considered to be knocked-down when it was seen lying on the floor of the wind tunnel and although movement was visible, it was unable to fly, even on gentle tapping of the floor underneath it. The mosquito was then carefully removed using an aspirator and placed in a paper cup. A piece of cotton wool soaked in 10% glucose solution was placed on the netting over the cup and the cups were kept at $65\% \pm 5\%$ r.h. and $25 \pm 1^\circ\text{C}$. Mortality was scored after 24 hours.

5.2.6 Netting Treatments

Pieces of nylon or cotton netting, 36 cm x 30 cm, were impregnated as specified in Chapter 2. The treatments are given in Table 5.

After the netting was dry, each piece was glued into a cardboard frame so that 30cm x 24cm of netting was exposed. The netting plus cardboard "window" for each experiment was placed in an aluminium frame inserted into the wind tunnel across the wind line. The polythene tubing was taped to the aluminium frame so that mosquitoes could only contact the area of netting placed across the wind tunnel, the sides and floor of the wind tunnel or the netting at the downwind end of the tunnel.

5.2.7 Recording

Mosquito activity was filmed from above the wind tunnel using a black and white Aqua SM72 video camera, and recorded on a JVC VHS portable Video cassette recorder (BR-6800).

A For A VTG-22 Video Timer set with a For A VTG-22 Stopwatch adapter was used to put the time in hundredths of a second on each frame of the video recording. The timer was started when the door to the entry tube was opened.

The mosquitoes were observed on a Hitachi monochrome Video monitor.

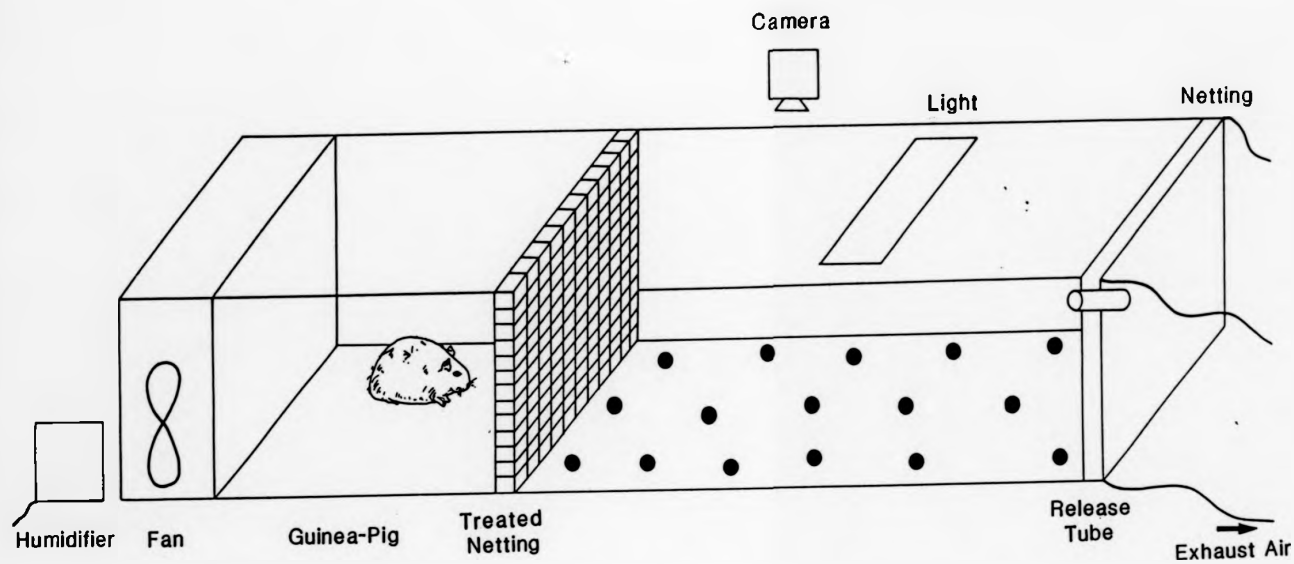


Figure 5(a) Wind Tunnel

Table 5

Netting Treatments for Wind Tunnel Study

<u>Species</u>	<u>Fabric</u>	<u>Treatment</u>	<u>Code</u>
<u>An. gambiae</u>			
"	Nylon	water (control)	C
"	Nylon	50mg permethrin/m ²	P50
"	Nylon	400mg permethrin/m ²	P400
"	Nylon	1000mg permethrin/m ²	P1000
"	Cotton	50mg permethrin/m ²	PC50
"	Cotton	1000mg permethrin/m ²	PC1000
"	Nylon	50mg permethrin + polystyrene/m ²	PS50
"	Nylon	400mg permethrin + polystyrene/m ²	PS400
"	Nylon	1000mg permethrin + polystyrene/m ²	PS1000
"	Nylon	200/200mg ² permethrin + pirimiphos-methyl	MIX
"	Nylon	400mg pirimiphos-methyl/m ²	PM400
"	Nylon	2mg lambdacyhalothrin/m ²	L2
"	Nylon	6mg lambdacyhalothrin/m ²	L6
"	Nylon	25mg lambdacyhalothrin/m ²	L25
<u>Cx. quinquefasciatus</u>			
"	Nylon	400 mg permethrin/m ²	CXP400
"	Nylon	25mg lambdacyhalothrin/m ²	CXL25

5.2.8 Analysis of recordings

The videos were played back using a JVC VHS Stereo Video Cassette Recorder (BR-6600E) and viewed on a JVC monitor.

Behaviour was divided into three activities: at 'Rest' on the netting (includes probing), 'Active' on the netting (includes walking and probing), and 'Off' the netting (includes flying, standing on the wind tunnel floor and on the netting at the end of the tunnel). The duration of each bout of activity was recorded to the nearest 20 milliseconds for every mosquito in all the treatments using the shuttle search and stop frame facility of the VCR. The results were analysed using a Nanostat program written by G. Gibson. For each treatment, the mean and standard deviation of the following were calculated:

Total time to k.d. or end of experiment whichever came first;

Total time spent resting on netting;

Total time spent active on netting;

Total time spent off the netting;

Total time on netting (i.e. resting + active);

Maximum duration of a resting bout;

Maximum duration of an active bout;

Maximum duration of a bout off the netting;

Number of resting bouts;

Number of active bouts;

Number of bouts off the netting;

Length of first resting bout;

Length of first active bout;

Length of first bout off the netting.

From the standard deviations, 95% confidence limits were calculated.

5.2.9 Lethal times

The methodology and calculation of the time taken to pick up a lethal dose is shown in section 4.6

5.3 RESULTS

5.3.1 Mortality

Table 5.1 shows that all the netting treatments caused 100% mortality, with the exception of the control (netting dipped in water only), P400 with Cx. quinquefasciatus and the two cotton treatments.

5.3.2 Knock-down

From Figure 5 (b), it can be seen that only the treatments incorporating medium and high doses of permethrin caused knock-down before the end of the exposure period of 10 minutes. The incorporation of polystyrene into the formulation did not significantly affect the time to knock-down or the mortality rate. On P400 Cx. quinquefasciatus mosquitoes were knocked down to the same degree as An.gambiae.

5.3.3 Irritancy

The presence of insecticides on the netting affected the behaviour of mosquitoes in several ways. The control mosquitoes generally landed on the netting and remained on it for the entire observation period. This was also observed with the mosquitoes which came into contact with the pirimiphos-methyl treated net. All the insecticide treatments affected the following aspects of behaviour to varying degrees:

- (1) proportion of time away from the netting (Figure 5 (c));

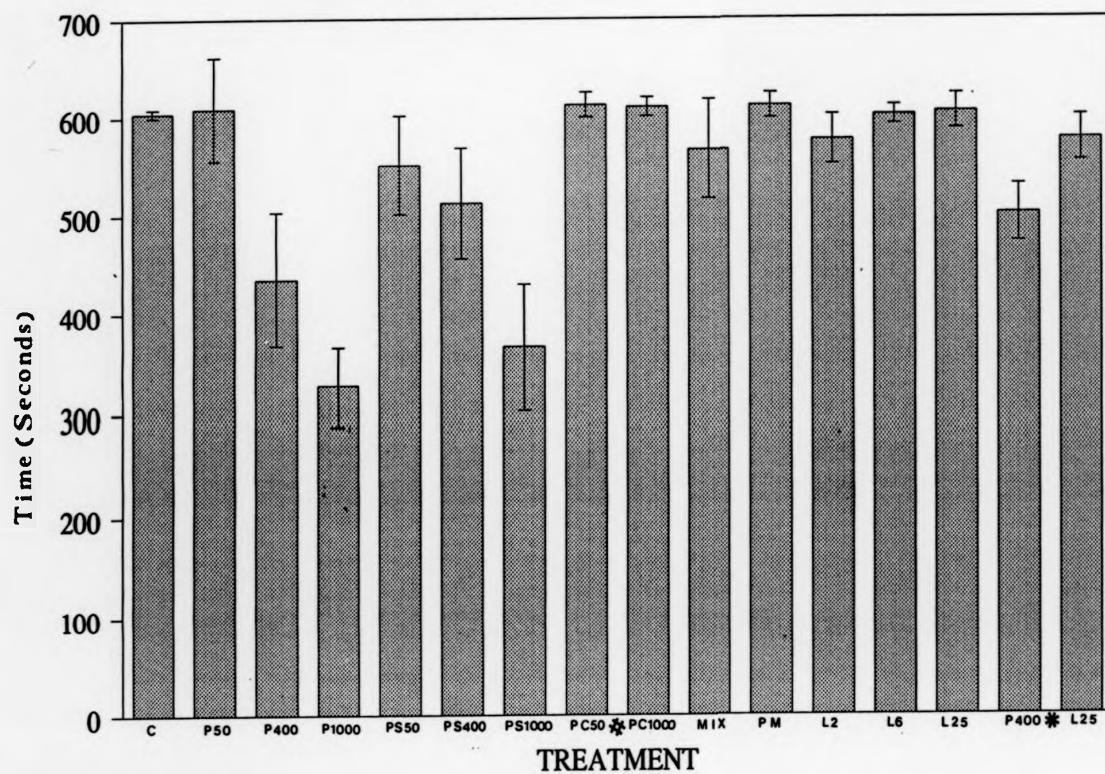
Table 5.1

Mortality and knock-down of An.gambiae and
Cx.quinquefasciatus after contact with netting treatments

NETTING TREATMENT	Time to K.D./ End of Expt $\bar{x} \pm 95\%$ c.l.* (seconds)	No. mosquitoes	No. dead (24 hrs)	Percent Mortality
<u>An.gambiae</u>				
C	604 \pm 4	21	1	4.8
P50	608 \pm 50	17	17	100
P400	435 \pm 68	15	15	100
P1000	327 \pm 38	22	22	100
PS50	550 \pm 50	16	16	100
PS400	512 \pm 56	14	14	100
PS1000	366 \pm 64	17	17	100
PC50	611 \pm 12	15	1	6.7
PC1000	609 \pm 9	16	9	56.3
MIX	566 \pm 50	16	16	100
PM400	610 \pm 12	14	14	100
L2	576 \pm 25	18	18	100
L6	601 \pm 9	21	21	100
L25	604 \pm 17	20	20	100
<u>Cx.quinquefasciatus</u>				
CXP400	501 \pm 29	16	14	87.5
CXL25	577 \pm 23	17	17	100

*Mean \pm 95% confidence limits

Figure 5 (b) Time to knock-down or end of experiment



Mean \pm 95% confidence limits
 n = 14 - 22, An.gambiae except *
 All treatments on nylon except #

- (2) correlation between contact time and bouts off the netting (Figure 5 (d) and 5(e));
- (3) proportion of time in contact with the netting at rest vs walking (Tables 5.2 and 5.3 and 5.4);
- (4) total contact time (Tables 5.2, 5.3 and 5.4);
- (5) Mean duration of each contact with the netting (Tables 5.2, 5.3, 5.4)

One of the most direct measures of irritancy was the percentage of time spent away from the netting. The higher doses of permethrin caused knockdown, so percentage of the observation time for which the mosquito was away from the netting has been used instead of absolute time. Figure 5 (c) shows that all insecticide treatments caused mosquitoes to spend a significantly greater percentage of time away from the netting than the control, with the exception of pirimiphos-methyl on its own. For permethrin, the percentage of time spent away from the netting increased with deposit density from 24% to 72%. This dose-dependent effect was lost by the addition of polystyrene to permethrin and the polystyrene reduced the percentage of time spent away from the netting at all doses to a mean of $23 \pm 10\%$. The response to lambdacy-halothrin was similarly not dose-dependent and the mean percentage of time spent away from the netting was $18 \pm 8\%$.

The mixture of permethrin and pirimiphos-methyl (200 mg/m^2 of each insecticide) caused the mosquitoes to spend approximately half the time spent away from the P400 treatment.

Both cotton treatments caused the mosquitoes to spend much lower percentages away from netting than their corresponding nylon treatments.

Cx. quinquefasciatus spent a lower percentage of time away from P400 than An.gambiae. However the converse was true with the L25 netting: in this case Cx. quinquefasciatus spent much more time away from the net (c.65%) than did An.gambiae (c.25%).

Although percentage of time spent away from the netting provides a rough estimate of degree of irritancy, more information about the effect of insecticides can be derived if we consider how the mosquitoes partitioned their time between contact with the net, and staying away from it. As Figures 5 (d) and 5 (e) show, there were, broadly speaking, four possible responses to treated netting:

- (A) mosquitoes could spend much time on the netting, with frequent changes in activity between resting, walking and flying,
- (B) they could change activities rarely, and spend most of their time in contact with the netting,
- (C) they could change activities rarely, and spend most of their time away from the netting,
- (D) they could switch activities often with a large number of bouts off the netting of long duration, with correspondingly short contacts with the netting.

Figures 5 (d) and 5 (e) show that the with the insecticide treatments used the mosquitoes tended to show roughly the same sort of activity switching. The degree of net contact was the behavioural factor which differed between treatments.

With the control netting, the mosquitoes spent most of their time on the netting, rarely leaving it.

5.3.3.1 Permethrin on nylon treatments

Figure 5 (d) shows that, as the dose increased, the percentage of time spent in contact with the netting by the mosquitoes significantly decreased. However, the number of bouts spent away from the netting was only significantly different between P50 and P1000.

There was no difference in the proportion of time spent at rest on the netting, compared to walking on the netting, between the three treatments. However, the total contact times showed a strong dose-dependency due to knock-down by the two higher

deposit densities (Table 5.2). The duration of the first resting bout also decreased with increasing dose although the 95% confidence limits of P400 and P50 overlap.

It is interesting to note that even at the highest deposit density, when mosquitoes were in contact with the net for only 28% of the time, before knock-down occurred they had picked up enough insecticide to cause 100% mortality. Table 5.2 shows that the contact time was approaching the minimum required to pick up a lethal dose, as the deposit density on the net increased. Table 5.5 shows that as the applied deposit density increased, the effective dose ($= \text{deposit density (g/m}^2) \times \text{contact time (seconds)}$) did not increase proportionally. Between P50 and P400 there was an 8 fold increase in concentration, the contact time decreased 2 fold, so the insects only received a 4 fold increase in exposure to insecticide. From P400 to P1000 there is a dose increase of 2.5 fold. The contact time decreased 2.5 fold, so the effective dose remained the same. If irritancy increased proportionally with deposit density, we might have expected the mosquitoes to spend less time at the highest deposit density.

There was no difference in the lengths of the first and maximum resting bouts implying that the mosquitoes did not become sensitized to, or tolerant of, the permethrin.

5.3.3.2 Permethrin on Cotton

Figure 5 (d) shows that the two permethrin-cotton treatments show a dose-dependent response to contact time as percentage of total time, but their confidence limits overlap on the number of bouts off the netting. This is explained by the fact that the length of time spent away was much longer for PC1000. Mosquitoes coming into contact with the cotton treatments spend much longer on them than they do on the corresponding nylon treatments. The mean resting times with the PC50 did not differ from the mean resting times with the control netting (Table 5.4).

5.3.3.3 Polystyrene/permethrin (wash-resistant) treatments on nylon

As Figure 5 (d) shows, the results with the three polystyrene treatments are similar to those with the PC and P50 treated netting. Thus addition of polystyrene to the permethrin formulation increased the total time spent by mosquitoes in contact with the netting but did not affect the proportion of bouts spent away.

5.3.3.4 Lambdacyhalothrin on nylon

In Figure 5 (e) it can be seen that all three lambdacyhalothrin treatments are clustered in the area of the graph associated with a high percentage of time in contact with the netting. They caused similar behaviour to the P50 treated netting. L25 shows a similar number of bouts off the netting as P400 and P1000, i.e. there is some evidence of irritancy developing at this, the highest, dose. The mean times spent resting, active on and off the netting were not significantly different in the three cases (Table 5.3). The proportion of resting time vs active time were observed to decrease with increasing dose but the differences were not significant.

The effective dose increased because total contact time did not change (Table 5.6) as there was no knockdown and little irritancy. The length of the first resting bout was not different from the length of the maximum resting bout (Table 5.3), i.e. the mosquitoes did not become tolerant of the pyrethroid.

5.4 DISCUSSION

100% mortality would be expected with the higher deposit densities of permethrin and lambdacyhalothrin used in these experiments but it is interesting to note that, after only 10 minutes exposure, both 50mg/m² permethrin and 2mg/m² lambdacyhalothrin gave 100% mortality also. These concentrations were used to simulate decayed field treatments, as well as to study dose dependency. The standard 3 minute bioassay of P50 treated nylon netting in a bioassay tube gave approximately 35% mortality

after 24 hours (section 4.1). Thus it can be seen that in more "natural" conditions, where the mosquito is allowed to try to obtain a blood meal, there is mortality at a deposit density which would previously have been viewed as too low to kill significant numbers. Although the insects became irritated by the permethrin they only spent about 23% of the time away from the netting, i.e. approximately 8 minutes exposure to 50mg/m^2 caused 100% mortality. The criterion of "effective dose" can help to explain this because at low deposit densities the mean total contact with the net is of sufficiently long duration to allow a lethal dose to be picked up despite this low deposit density of insecticide. Likewise, "effective dose" may explain the finding of Lines *et al.* (1987) that increasing the deposit density of permethrin on nylon bednets or curtains from 200mg/m^2 to 1000mg/m^2 did not cause the reductions from the untreated control values found in blood-feeding or mortality to differ significantly. The present studies found that increasing the deposit density of permethrin from 400mg/m^2 to 1000mg/m^2 (i.e. an increase of 2.5) caused a decrease in mean contact time with the netting of 2.5 so that the effective dose picked up by the mosquitoes was the same at these two deposit densities.

With the lambdacyhalothrin treated netting, because the total contact times do not vary with dose, the effective dose increases with increasing deposit density of insecticide on the netting. It would be very interesting to decrease the deposit densities of permethrin and lambdacyhalothrin still further in order to determine the lowest deposit densities of each which still caused 100% mortality under these test conditions. The permethrin cotton treatments were used to compare the mortality, knock-down and irritancy with the same nylon treatments. Since PC50 did not cause any significant mortality in the 10 minute test, a higher deposit density (PC1000) was tested and found to cause 56% mortality. It might be of interest to leave the insects until knock-down occurred on these treatments to see how long it takes for higher mortality to be achieved.

In the 10 minutes of recording, only the permethrin treatments on nylon

(excluding P50) caused the insects to be knocked down. Lambdacyhalothrin belongs to the type II pyrethroids which cause poorer knock-down but better kill than the type I (e.g. permethrin). Spinney (pers. comm.) found, in a comparison of wettable powder formulations commonly used in vector control, that the permethrin (200mg/m^2) treated surface caused significantly quicker knock-down of An.gambiae s.s than a surface treated with $30\text{ mg lambdacyhalothrin/m}^2$. He also found that the proportion of time spent by mosquitoes in contact with a pirimiphos-methyl treated surface (1000mg/m^2) was not significantly different from the control however, the corresponding times of lambdacyhalothrin and permethrin treated surfaces differed significantly.

The percentage of time spent away from the netting is related to the irritancy of the insecticide/fibre combination. With a host-seeking mosquito there will be a conflict between attraction to a blood-meal and repulsion from the insecticide.

There was no difference in the percentage of time spent away from the netting with the three polystyrene deposit densities. This may have been due to a masking effect caused by the polystyrene. The observed percentages of time spent away were lower than the ordinary permethrin on nylon treatments for the 400 and 1000 mg/m^2 deposit densities (the 95% c.l.s overlapped in the case of the 50 mg/m^2) which again may have been attributable to this masking effect. It is interesting to note that time to k.d. was not affected by the addition of polystyrene. Although it might be supposed that less insecticide would be picked up from the netting treated with permethrin alone (greater time spent away from the netting), it may be that the polystyrene is causing some kind of a barrier to pick-up; therefore k.d. does not occur more quickly than with ordinary permethrin.

The treated cotton netting caused mosquitoes to become less irritated than the treated nylon netting. This was also found using the actograph to assess flight activity as a measure of irritancy (4.5). The wind tunnel method of assessing the irritancy caused by insecticide treated netting is more sensitive than using an actograph since it allows the mosquitoes to move away from the treated netting. The actograph

essentially compares the effects of treatments on mosquitoes forced to contact them and in this respect is more like the WHO tube test. With both permethrin and lambdacyhalothrin the lengths of the maximum resting times were not significantly different to the duration of the first resting contacts. This implies that the mosquitoes became sensitized to the treatments and not habituated to them.

Cx. quinquefasciatus were very much more irritated by the L25 than An. gambiae. The reason for this is unclear, but the result highlights the fact that the susceptibility to behavioural effects of insecticides are not always closely correlated with those of lethal effects. However, since all the Culex quinquefasciatus died, there is no reason to suppose that the irritancy would prevent killing under field conditions.

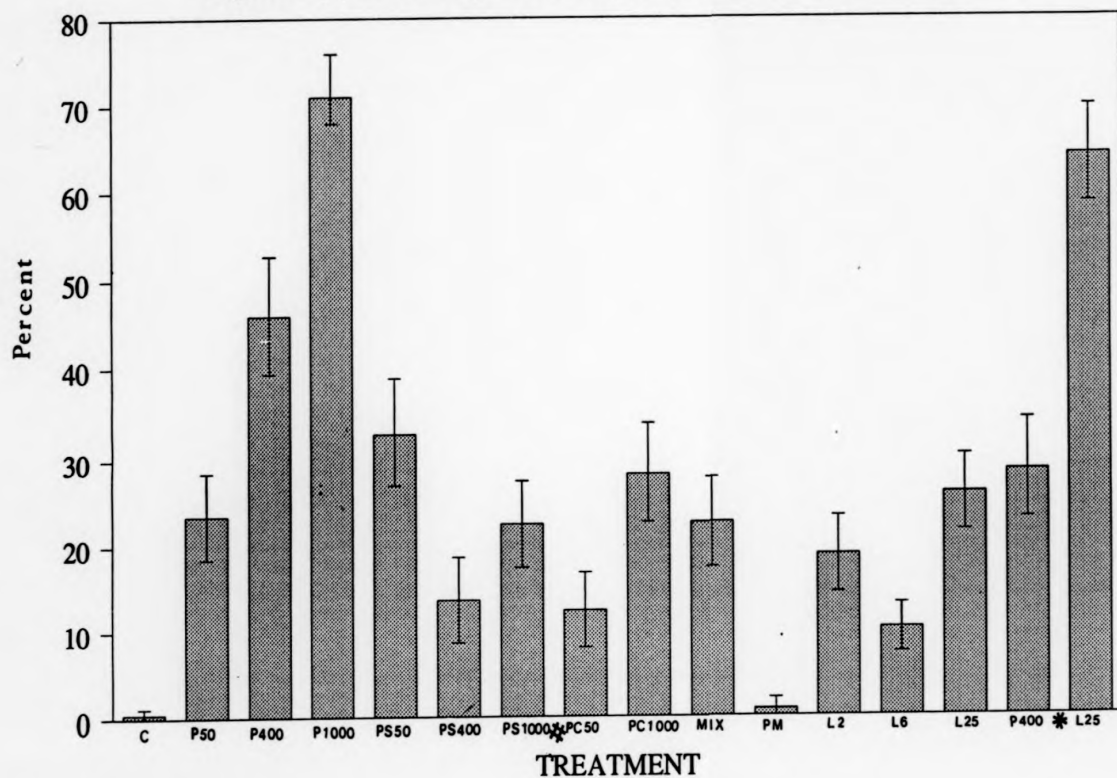
The final question addressed was: how long an exposure was required for the mosquitoes to pick-up a lethal dose? In the case of the permethrin treatments, the time before knock-down was always sufficient to kill 100% of An. gambiae. However from Table 5.2 it can be seen that the total time spent resting and active on the netting corresponded to the LT95 value (determined as specified in 4.6) in the case of P50 and P400, and the LT90 value in the case of P1000. Thus mosquitoes in the wind tunnel experiments died after slightly lower netting contact times than expected from the LT99 determined as specified in 4.6). With the opportunity to behave naturally and the stress of flying in the wind tunnel, it appears that they were more susceptible to insecticide treated netting than when exposed to it under the controlled conditions reported in 4.6. It therefore seems likely that, in the field, greater mortality would occur after a given contact time than that obtained with the LT tests (4.6).

With the lambdacyhalothrin treatments, Table 5.3 shows that the first resting bout corresponded to the LT95 values in the case of L2 and L6 and the LT99 value for L25. Since knock-down did not occur in the 10 minutes of the experiment, the mosquitoes actually received more than enough insecticide to kill them on the lambdacyhalothrin treated netting.

5.5 SUMMARY of Chapter 5

- (1) With these treatments irritancy did not reduce mortality since in the two cases where mortality was significantly less than 100%, less than 30% of time was spent away from the netting.
- (2) Knock-down of mosquitoes only happened with the permethrin treatments.
- (3) Irritancy was most significant with the permethrin treatments and its significance was reduced by mixing permethrin with polystyrene or pirimiphos-methyl, although mortality was not affected by this.
- (4) The type of irritancy induced by permethrin has the effect of decreasing the number and length of contacts on treated netting as deposit density increases and of increasing the total time away from the netting. Lambdacyhalothrin increases the number of contacts and reduces the duration of the contacts, so that total contact time is not decreased.

Figure 5 (c) Percentage of time spent away from the netting



Mean \pm 95% confidence limits

n = 14 - 22, *An.gambiae* except * *Cx. quinquefasciatus*

All treatments on nylon except #

Figure 5 (d) Number of bouts off the netting (as % of total bouts) , versus percentage of total time spent in contact with netting, permethrin and control

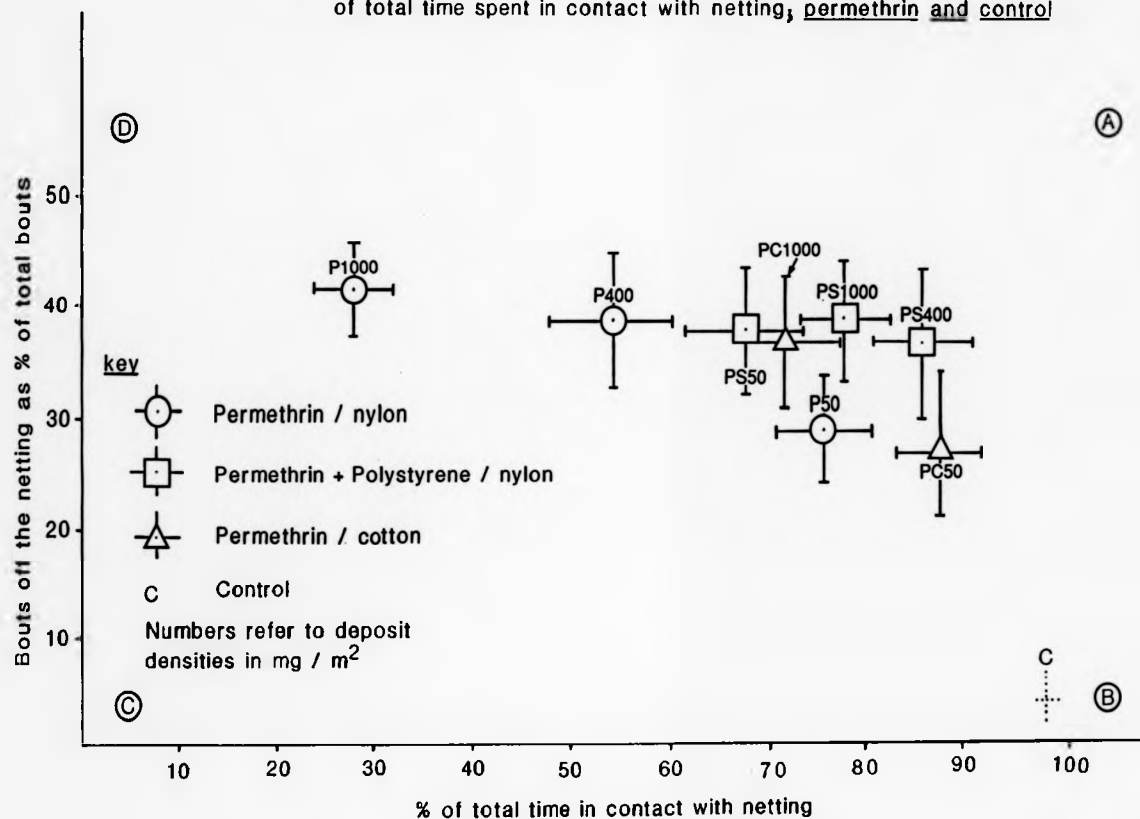


Figure 5 (e) Number of bouts off the netting (as % of total bouts), versus percentage of total time in contact with netting; permethrin, lambdacyhalothrin and control

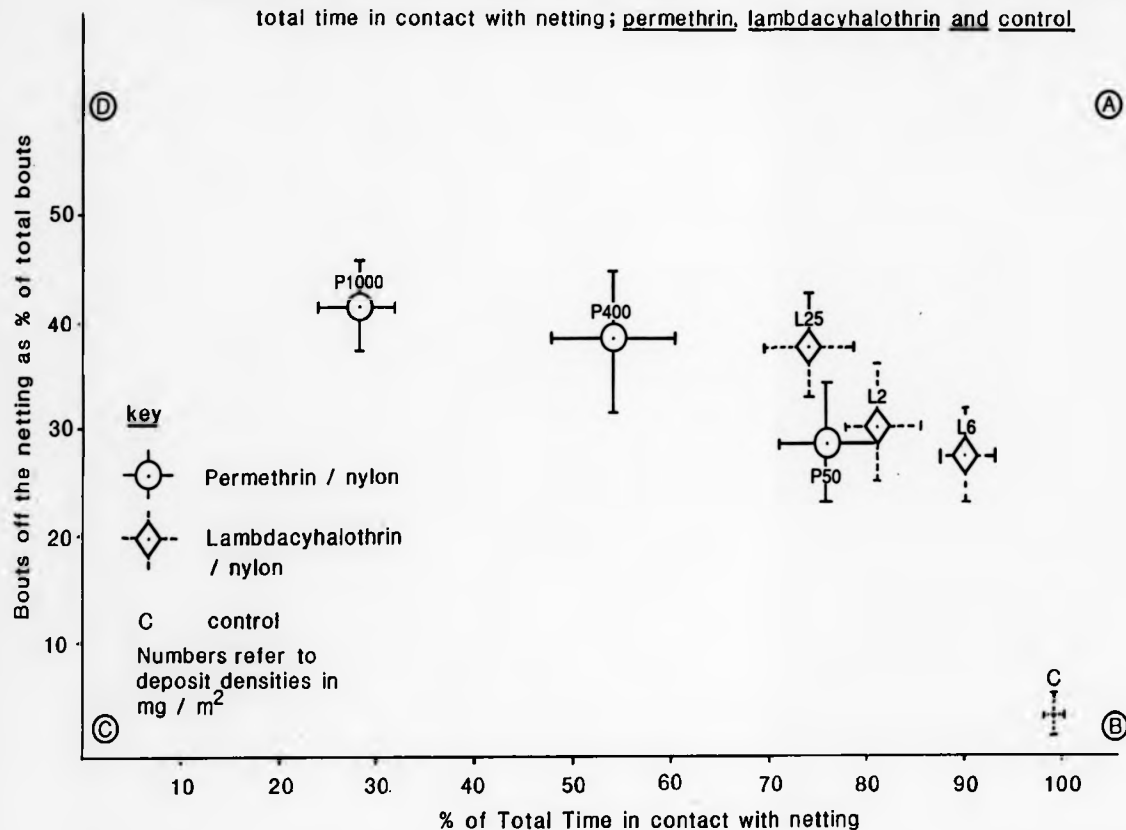


Table 5.2

Activity and Lethal times (LT values) of An.gambiae on permethrin treated netting.

All times in seconds.

ACTIVITY	P50	P400	P1000
Total contact time (mean \pm 95% c.l.)	465 \pm 72	235 \pm 37	92 \pm 20
Time of first resting bout ($\bar{x} \pm$ 95% c.l.)	70 \pm 18	59 \pm 29	18 \pm 15
Time of maximum resting bout ($\bar{x} \pm$ 95% c.l.)	75 \pm 16	64 \pm 27	19 \pm 8
LT 90	395	175	91 *
LT 95	453 *	247 *	134
LT 99	586	471	282
Proportion of total time in contact with net (\pm 95% c.l.)	0.76 \pm 0.08	0.50 \pm 0.12	0.28 \pm 0.04
Proportion of contact time resting vs active. (\pm 95% c.l.)	0.24 \pm 0.05	0.32 \pm 0.06	0.22 \pm 0.04
Mean (\pm 95% c.l.) time of each bout:			
Resting on net	97 \pm 30	47 \pm 26	47 \pm 18
Active on net	70 \pm 30	39 \pm 10	64 \pm 20
Off the net	97 \pm 30	63 \pm 14	95 \pm 20

* consequence of total contact time with netting (see text)

Table 5.3

Activity and Lethal times (LT) of An.gambiae on
lambdacyhalothrin treated netting

All times in seconds.

ACTIVITY	L2	L6	L25
Total contact time (mean \pm 95% c.l.)	468 \pm 39	539 \pm 36	447 \pm 46
Time of first resting bout (\bar{x} \pm 95% c.l.)	105 \pm 42	106 \pm 38	66 \pm 27
Time of Maximum resting bout (\bar{x} \pm 95% c.l.)	117 \pm 44	78 \pm 73	80 \pm 27
LT 90	67	46	9
LT 95	97*	60 *	12
LT 99	194	98	21 *
Proportion of total time in contact with net (\pm 95% c.l.)	0.81 \pm 0.04	0.90 \pm 0.03	0.74 \pm 0.04
Proportion of contact time resting vs active. (\pm 95% c.l.)	0.27 \pm 0.05	0.19 \pm 0.04	0.18 \pm 0.04
Mean \pm 95% c.l time of each bout:			
Resting on net	106 \pm 42	105 \pm 38	57 \pm 20
Active on net	193 \pm 62	187 \pm 82	112 \pm 30
Off the net	56 \pm 22	31 \pm 16	46 \pm 10

* consequence of first resting bout (see text)

Table 5.4

Activity times of *An.gambiae* on control, pirimiphos-methyl (PM), pirimiphos-methyl/permethrin (Mix) treated nylon netting and cotton netting treated with permethrin.

All times in seconds.

PC = Cotton netting treated with 50mg permethrin /m²

PC1000 = Cotton netting treated with 1000mg permethrin/m²

Activity	Control	PM	Mix	PC50	PC1000
Proportion of total time in contact with netting (± 95% c.l.)	0.99 ± 0.04	0.99 ± 0.01	0.77 ± 0.05	0.87 ± 0.05	0.72 ± 0.06
Proportion of contact time resting vs active (± 95% c.l.)	0.81 ± 0.04	0.41 ± 0.07	0.34 ± 0.06	0.48 ± 0.07	0.28 ± 0.06
Mean ± 95% c.l time of each bout:					
Resting on net	156 ± 42	154 ± 28	66 ± 8	147 ± 44	74 ± 19
Active on net	21 ± 5	219 ± 32	90 ± 19	125 ± 24	107 ± 21
Off the net	1.7 ± 0.7	3.7 ± 2.8	48 ± 15	51 ± 25	63 ± 12

Table 5.5. Effective dose from permethrin impregnated netting.

Treatment	Number of contact bouts.	Mean contact (seconds)	Deposit density (mg/m ²)	Effective Dose (g m ⁻² s)
P50	9.06	465	50	23,250
P400	5.73	235	400	94,000
P1000	3.68	92	1000	92,000

Table 5.6 Effective Dose from lambdacyhalothrin impregnated netting.

Treatment	Number of contact bouts.	Mean contact (seconds)	Deposit density (mg/m ²)	Effective Dose (g m ⁻² s)
L2	3.83	468	2	986
L6	2.95	539	6	3,234
L25	5.60	447	25	11,175

CHAPTER 6
EXPERIMENTAL HUT TRIALS OF BEDNETS IMPREGNATED WITH
INSECTICIDES FOR MOSQUITO CONTROL IN THE GAMBIA,
1988 and 1989

6.1 Trial in 1988 of cypermethrin, deltamethrin, lambdacyhalothrin,
permethrin and pirimiphos-methyl

6.1.1 INTRODUCTION

At present the insecticides which have been chosen for the impregnation of bednets are the synthetic pyrethroids, permethrin and deltamethrin, which have been used in studies in many parts of the world (see Introduction). Until recently only the two pyrethroids, permethrin and deltamethrin, had been evaluated in the field and direct comparisons between them had not been made in the same location. A trial has now been implemented using lambdacyhalothrin on bednets in Tanzania (Lines *et al.*, in prep.). Other insecticides tested on bednets are the organophosphates, fenitrothion, chlorpyrifos-methyl, chlorphoxim and pirimiphos-methyl (Brun and Sales, 1976) and the organochlorine DDT, which was directly compared with deltamethrin (Li Zu-zi *et al.*, 1987).

In most studies impregnated bednets have remained unwashed. In The Gambia, women wash nets regularly in the rainy season, often at fortnightly intervals (Snow *et al.*, 1987 [a]). Nets are vigorously washed by hand in cold water, using locally made soap. Washing in this fashion greatly reduces the concentration of synthetic pyrethroid on fabric as assessed by bioassays and gaschromatography, G.C., (Snow *et al.*, 1987 [a]; Lindsay *et al.*, in press).

The present trial had two aims. Firstly, to compare the insecticidal activity of four pyrethroids, cypermethrin, deltamethrin, lambdacyhalothrin and permethrin and the organophosphate pirimiphos-methyl, using experimental huts. The pyrethroids were selected from earlier comparisons of netting impregnated with different insecticides (Lindsay *et al.*, in press, and Miller, submitted). The target deposit

densities of permethrin and deltamethrin were those used in The Gambia (Snow *et al.*, 1987 [a] and [b]), and China (Lu Zu-zi *et al.*, 1987) respectively. The target deposit densities of cypermethrin and lambda-cyhalothrin were those estimated to be equitoxic with the other insecticides based, on values given by Herve (1985). The organophosphate was included in order to compare its performance with the pyrethroids and with a view to formulating mixtures of insecticides as a possible strategy to delay the evolution of pyrethroid resistance (Curtis, 1987). The second aim was to assess the effect of hand-washing on persistence and activity of the above insecticides on bednets.

6.1.2 METHODS AND MATERIALS

6.1.2.1 Bednets

The bednets were made from nylon netting with a 1 mm mesh and weight of 31g/m². They were 2.2m long, 0.95m wide and 1.8m high, with a total area of 13.43 m². To simulate badly torn bednets, six pieces measuring 10 by 10 cm were cut from each net, making holes, two at either side and one at each end.

6.1.2.2 Insecticides

The target deposit densities of each insecticide were as follows:

cypermethrin - 100mg/m² (from 10% emulsifiable concentrate E.C., "Cymperator", I.C.I.),

deltamethrin - 25mg/m² (from 2.5% E.C., "Decis", Roussel Uclaf),

lambda-cyhalothrin - 25mg/m² (from 2.5% E.C., "Icon", I.C.I.),

permethrin - 500mg/m² (from 25% E.C., "Imperator", I.C.I.)

pirimiphos-methyl - 1000mg/m² (from 50% E.C., "Actellic", I.C.I.).

6.1.2.3 Study Area

The study was carried out at Wali Kunda, a field station in an area of Sudan savanna on the south bank of the River Gambia, approximately 290km from the coast (Figures 6.1 (a) and 6.1 (b)). This site is bordered on the north by the fast-flowing freshwater river. The main mosquito breeding sites are the rice fields and swampland to the south, covering approximately 610 hectares. The huts were situated in an area of open grassland with scattered trees.

6.1.2.4 Experimental Huts

Six experimental huts (Plate 4 (a)) were built in a line, 12.00 metres apart, parallel to the edge of the large area of rice and swamp. The design and dimensions were similar to those described by Rapley (1961) and Smith (1965), based on mud walled and thatch roofed African houses with verandahs extending from each of the four walls. The eaves were open and the verandahs screened with mosquito netting on the two sides of the hut perpendicular to the edge of the rice swamp. This screening was permanently fixed and so the periodic exchange of screened for unscreened verandahs (Smith, 1965) to correct for possible selective exit of mosquitoes in one direction, could not be duplicated with these huts. Window traps were placed over the windows on the two walls of the screened verandahs. The other two windows were closed. Each hut was raised from the ground on concrete pillars which had water channels around them (Plate 4 (b)). These moats prevented the entry of ants and termites which would have removed the corpses of dead mosquitoes in the night.

6.1.2.5 Impregnation of Bednets

The volume of water a bednet absorbed was determined by weighing the net dry, saturating it in distilled water for 5 minutes, wringing out the excess water and re-weighing the net. This was carried out for 3 nets and a mean of 538 mls was found to be absorbed.

Figure 6.1(a) Wali Kunda Field Station

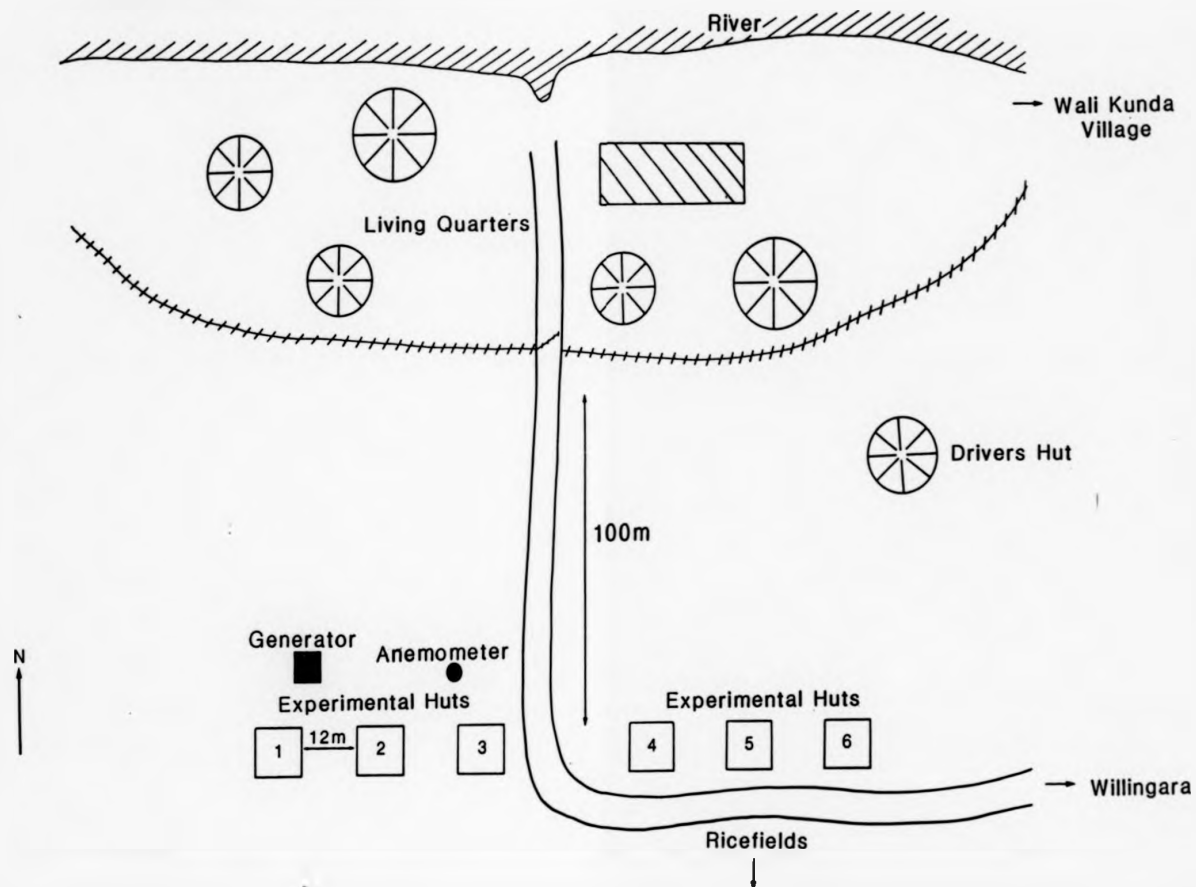
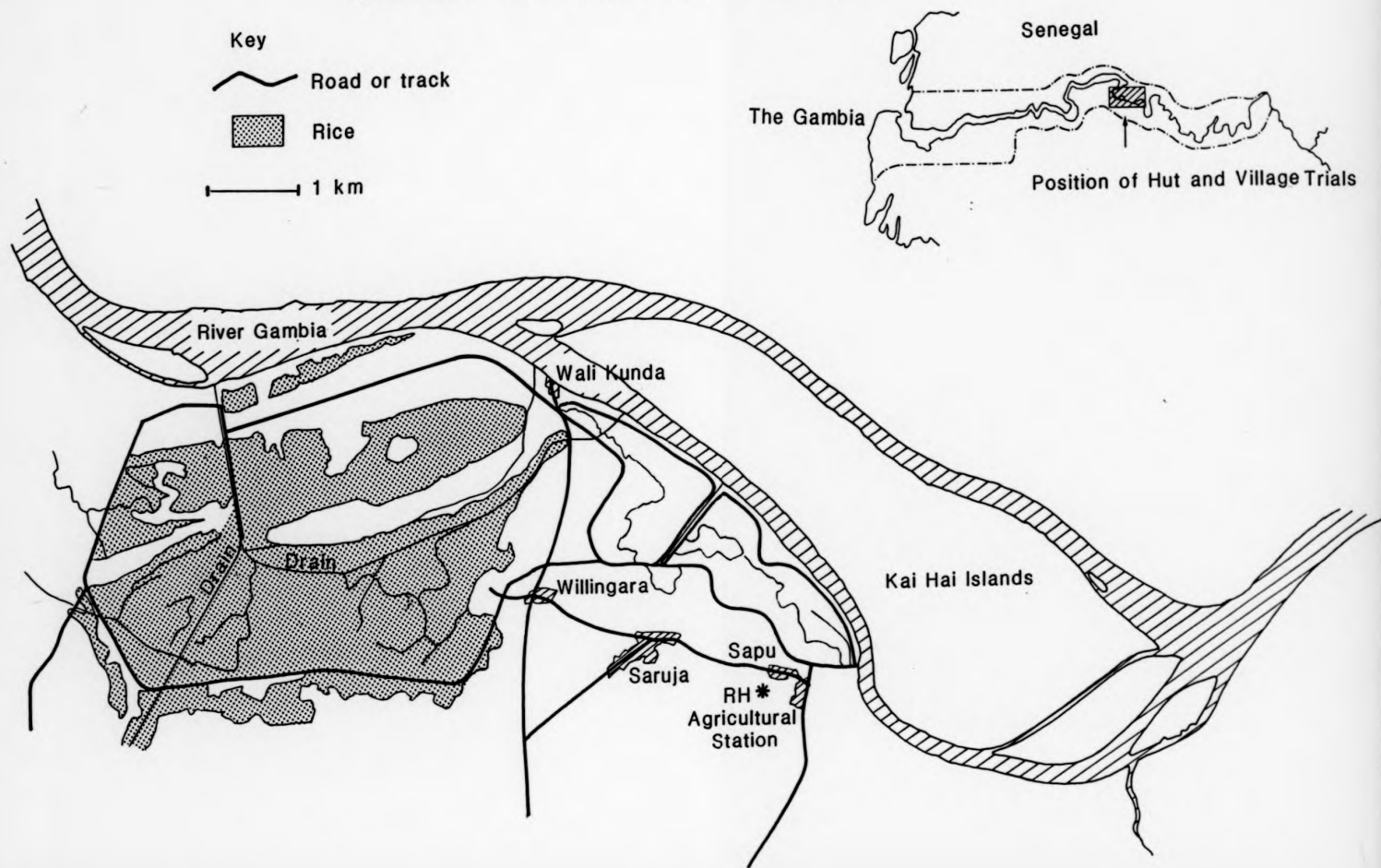


Figure 6.1(b) Wali Kunda, Suruja & Surrounding area.





Plates 4 (a) and (b) Experimental huts at Walikunda

The amount of insecticide required for each treatment was calculated using the following formula:

Amount of E.C.(mls) =

$$\frac{(\text{Target deposit density (mg/m}^2 \times \text{area of fabric (m}^2) \times 100}{\% \text{ of a.i. in the E.C.}}$$

The E.C. was added to water to make an emulsion of 538 mls.

Two nets were impregnated with each insecticide: each net was left for 5 minutes in the emulsion and then wrung out by hand. Two nets were dipped in water to act as controls. Nets were left to dry horizontally indoors on plastic sheeting for 24 hours and colour coded so that the trial could be carried out double blind.

6.1.2.6 Washing of Nets

At the start of the trial, one of each of the 6 pairs of nets was hand washed 3 times on 3 separate days by 3 different Gambian women. The nets were washed separately in river water using local "cow fat" soap. Each net was rubbed thoroughly washed for about 7 minutes and then rinsed in the river (Plate 5).

Nets were dried indoors to avoid possible U.V. degradation of the insecticides.

6.1.2.7 Chemical Analysis

To determine the amount and distribution of insecticide on each net, the pieces of material removed to make the holes in the nets were analysed using gas chromatography.



Plate 5 Washing nets for hut trials



Plate 6 Bioassaying nets in village trial

6.1.2.8 Sleepers

Six Gambian men, aged 18-55 years, slept in the huts each night for the duration of the trial. They were questioned every morning to determine any perceived side effects of sleeping under the treated bednets. Sleepers were given chloroquine prophylaxis weekly and paid.

6.1.2.9 Collection of mosquitoes

Each hut, with its enclosed verandahs and exit traps was searched using aspirators and electric torches for 60 person-minutes every morning. The mosquitoes were later identified and scored as fed or unfed. All Anopheles gambiae sensu lato caught alive were provided with 10% glucose and held in paper cups. Mortality rates were recorded after 24 hours. The window traps and verandahs were provided with 10% glucose solution to reduce the risk that unfed females exiting in the night would die of starvation.

An estimate of the total number of mosquitoes which entered the huts was made by doubling the verandah trap catches (since mosquitoes could also go out through the two open eaves) and adding this to the numbers found in the window traps and inside the huts (Smith, 1965). This method of estimating the number of mosquitoes which entered the hut assumes that none can escape through the roof etc (see Discussion 6.3). The same principle ($2 \times$ verandah + window traps + inside hut) was used to estimate the total number of mosquitoes killed.

6.1.2.10 Design of trial

The trial lasted six weeks, with a one night break at the end of each week (Saturday). Each sleeper slept from 22.00 to 06.30 hours in the same hut for six consecutive nights. Three Latin Square designs (Cochran and Cox, 1957) were used: (1) to allocate the six sleepers to a different hut each week, (2) to allocate the five

different insecticide treated nets and the untreated net to different sleepers on each of the six nights of the week and (3) to allow for systematic variation between nights of the week. In addition, the washed and unwashed nets were allocated by imposing washing on the second Latin Square, so as to be as nearly balanced as possible in each week (Appendix 6.1 (a)).

To summarize, 216 observations were made, one from each of the six huts on the 36 nights of the study. Each insecticide was tested 36 times.

6.1.2.11 Statistical analysis

The data for counts of mosquitoes were log transformed ($\ln x+1$) and the proportions angular transformed ($\arcsin \sqrt{x}$) to stabilize the variances. Analysis of variance (ANOVA) was carried out using the statistical software package GLIM (Payne *et al.*, 1986). The ANOVA allowed for variation between huts, nights, sleepers, treatments and for the effect of washing. To assess whether washing affected the insecticides in different ways, an "insecticide by washing" interaction term was also included in the model. The means given are adjusted for the night of the trial, since only six of the twelve treatments were tested on any one night (Appendix 6.1 (b)).

For the numbers of live bloodfed mosquitoes of each species, there was a high proportion of zero counts and these data could not be considered as normally distributed when log transformed. These responses were therefore considered as binary (mosquitoes found or not found) and logistic regression techniques applied. For simplicity, univariate results are presented here since although the night-to-night variation was significant, allowing for it did not alter the conclusions. Hut-to-hut and sleeper-to-sleeper variation was not significant, so a model including night-to-night variation and treatment was selected. χ^2 tests were carried out to compare the treatments (Appendix 6.1 (c)).

6.1.2.12 Identification of bloodmeals

Enzyme linked immunosorbant assay (ELISA) tests (double sandwich technique of Service *et al.* (1986)) were carried out (Appendix 6.1 (d)). In the present trial (6.1) only a sample (45%) of the blood meals of An. gambiae s.l. were identified. In the second trial (6.2) all the An.gambiae s.l. found bloodfed were analysed.

6.1.3 RESULTS

The mean actual deposit densities (mg/m^2) as assessed by G.C. are shown in Table 6.1.

The following response variables were analysed:

- (i) Total number of mosquitoes found in hut;
- (ii) Mosquito mortality; (a) total number of mosquitoes which were found dead in the hut, window traps and verandahs plus those dead after 24 hours; (b) percentage of the total number found in the hut, window traps and twice the numbers in the verandahs which were dead plus those which had died after 24 hours;
- (iii) Mosquito bloodfeeding: (a) total number found bloodfed in hut, windowtraps and twice the numbers in the verandahs; (b) percentage of the total number found in hut, window traps and twice the numbers in the verandahs which were bloodfed; (c) total number found alive and bloodfed in the hut, window traps and twice the numbers in the verandahs;
- (iv) Exiting: the percentage of the total number estimated to have entered (i.e numbers in hut, window traps and twice the numbers in the verandahs) which were found in the window traps and twice the number in the verandahs.

Anopheles gambiae s.l. and Mansonia spp. were considered separately. Of 425 Mansonia spp. examined 93% were found to be Mn. africana and 7% Mn. uniformis.

No sibling species identification of the An. gambiae s.l. was undertaken but preliminary cytotaxonomic identification of a limited number of specimens indicates that An. gambiae s.s. savannah type is the predominant species in this part of The Gambia in the wet season (G.White, pers. comm.).

The results of the ANOVAs for An. gambiae s.l. and Mansonia spp are shown in Tables 6.1 (a) and 6.1 (b) respectively. Where F values are significant, t-test comparisons between individual treatments are justifiable. For both mosquito species, the difference between insecticides was significant for all the responses investigated, with the exception of percentage exiting of An.gambiae s.l. and borderline significance for percentage of An. gambiae s.l. bloodfed. For Mansonia spp., the effect of washing and the way in which washing affected each insecticide was also significant (Appendix 6.1 (b)).

6.1.3.1 Total numbers of mosquitoes found in huts and traps.

The effect of bednets submitted to different treatments on the number of mosquitoes found in huts and their traps is shown in Figure 6.1.1. In this and all subsequent figures (except percentage of occasions when live blood fed mosquitoes were found) the means and approximate standard error of the difference between any two means (S.E.D.) are shown.

About 60% fewer An. gambiae s.l. were found in the hut (with its verandahs and window traps) with the unwashed permethrin treated net than in the hut with the unwashed untreated net ($t=4.9$, $d.f=30$, $p<0.001$). There were no significant differences between the numbers of An. gambiae s.l. with the other nets. Thus the permethrin net appears to have had a deterrent effect against mosquito entry but this deterrent effect of permethrin was lost after washing.

Significantly fewer Mansonia spp were found in the presence of the unwashed permethrin net compared with other unwashed nets (t-tests: $p<0.001$) and 68% fewer than with the unwashed, untreated net ($t=7.3$, $d.f=30$, $p<0.001$).

With the unwashed lambdacyhalothrin and pirimiphos-methyl nets significantly fewer mosquitoes were found in the huts (and their traps) than with unwashed untreated, cypermethrin or deltamethrin treated nets (t-tests: $p < 0.05$). Compared to the unwashed, untreated net, it was estimated that 37% and 32% fewer Mansonia spp. entered the huts containing the pirimiphos-methyl and lambdacyhalothrin treated nets, respectively. After washing, none of the insecticide treated nets reduced the entry rates of Mansonia spp.

6.1.3.2 Mosquito mortality.

The effect of different treatments on the total number of mosquitoes killed is shown in 6.1.2 (a) and the percentages of the mosquitoes entering the huts which were killed is shown in Figure 6.1.2 (b).

6.1.3.2 (a) Total number of mosquitoes killed:

All the unwashed, treated nets killed a significantly higher number of An. gambiae s.l. than the untreated one, although this was of borderline significance ($p \sim 0.05$) in the case of the permethrin treated net. After washing the nets, the total numbers of An. gambiae s.l. killed were reduced but all the nets, with the exception of the permethrin one, killed more mosquitoes than the untreated net. For Mansonia spp. all the insecticide treated nets, except the permethrin, killed a larger number of mosquitoes than the untreated net. The cypermethrin treated net killed the most Mansonia spp. (mean = 44.6 per night), but this number was not significantly different from the deltamethrin, lambdacyhalothrin or pirimiphos-methyl treated nets, which killed on average 35.5, 29.7 and 29.6 Mansonia spp. per night respectively.

After washing, all the treated nets killed more mosquitoes than the untreated net. The lambdacyhalothrin treated net killed the greatest number of Mansonia spp. (adjusted mean 40.9 per night), but this number was not significantly different from

the number killed by the pirimiphos-methyl treated net (adjusted mean 29.2 per night).

6.1.3.2 (b) Percentages killed:

For An. gambiae s.l. with the unwashed nets, all the insecticides caused significantly greater mortality than the unwashed untreated net. After washing, all the nets, except the permethrin treated one, remained significantly more insecticidal than the untreated net (t-tests: $p < 0.05$). For Mansonia spp., all the unwashed, insecticide treated nets caused significantly higher mortality than the unwashed, untreated net. The highest percentage of the Mansonia spp. entering the huts was killed with the pirimiphos-methyl net, and the mortality was significantly higher with this organophosphate than with all the pyrethroids except lambdacyhalothrin. The pirimiphos-methyl and lambdacyhalothrin nets killed 64% and 62% more Mansonia spp., respectively, than the untreated net. For the washed nets, only cypermethrin, pirimiphos-methyl and lambdacyhalothrin caused significantly higher mortality than the untreated net (t-tests: $p < 0.05$). The mortalities with these nets were 29%, 35% and 40% respectively.

6.1.3.3 Mosquito bloodfeeding:

The effect of different treatments on the total number bloodfed, percentage bloodfed and number of live bloodfed mosquitoes is shown in Figures 6.1.3 (a), (b) and (c).

The analyses are based on all mosquitoes found blood fed, regardless of the origin of the blood meal i.e. it included mosquitoes which had fed on animals outside and entered already fed.

6.1.3.3 (a) Total number bloodfed:

With An.gambiae s.l. there was no difference between the washed and unwashed treatments. The permethrin treated net was associated with the lowest number of bloodfed mosquitoes and the number differed significantly from that with the untreated net ($t = 3.88$, $d.f = 30$, $p < 0.001$). The lambdacythothrin net was the only other treated net to give results differing significantly from those with the untreated net ($t = 2.0$, $d.f = 30$ $p \sim 0.05$). With Mansonia spp. all the unwashed, insecticide treated nets reduced the total number of bloodfed mosquitoes, compared to the untreated net ($p < 0.001$). There were no significant differences between the deltamethrin, pirimiphos-methyl or cypermethrin treated nets. After washing this effect was lost.

6.1.3.3 (b) Percentage of bloodfed mosquitoes:

Although the unwashed, lambdacyhalothrin treated net was associated with the lowest observed percentage of bloodfed An. gambiae s.l., this was not significantly lower than with the unwashed untreated net.

For Mansonia spp. the unwashed, untreated net was associated with a significantly higher percentage of bloodfed mosquitoes than any of the other unwashed nets (t-tests: $p < 0.01$). The unwashed lambdacyhalothrin and permethrin treated nets were associated with the lowest percentages of bloodfed mosquitoes, with reductions of 86% and 91% respectively, compared to the untreated net. With washed nets, pirimiphos-methyl was associated with 46% more bloodfeds than the untreated net and was the only net to differ significantly from the untreated net in this respect.

6.1.3.3 (c) Total numbers of live bloodfed mosquitoes:

For An.gambiae s.l., the percentage of observations where live bloodfed mosquitoes were found was lower for all the unwashed and washed insecticide treated

nets than for the unwashed, untreated net. On every occasion (i.e. 18 nights) when the unwashed, pirimiphos-methyl net was used, no live bloodfed An. gambiae s.l. were found.

The percentage of observations where live, bloodfed Mansonia spp. was found was lower for all the unwashed, insecticide treated nets than for the unwashed, untreated net.

The percentage of live bloodfeds was lower for the washed lambdacyhalothrin, cypermethrin and deltamethrin treated nets than for the washed, untreated net.

6.1.3.4 Percentage Exiting:

The percentages of mosquitoes which had exited are shown in Figure 6.1.4.

For the unwashed nets, the percentage of An. gambiae s.l. exiting was significantly lower ($p < 0.01$) with deltamethrin, cypermethrin and pirimiphos-methyl than with the untreated net. However, with the washed nets, the insecticide treated nets were all associated with higher observed percentages of mosquitoes exited, although the difference was only significant in the case of the permethrin treated net ($t = 2.2$, $d.f = 30$, $p < 0.05$).

The unwashed pirimiphos-methyl, permethrin and lambdacyhalothrin treated nets caused significantly lower ($p < 0.01$) percentages of Mansonia spp. to exit than the untreated net, but among the washed nets, only that treated with lambdacyhalothrin caused a significantly lower percentage to exit than the untreated net ($t = 3.3$, $d.f = 30$, $p < 0.01$).

6.1.3.5 Blood meal identification

ELISA tests of 705 An. gambiae s.l. blood meals showed that 59.1% of the mosquitoes had fed on humans and 40.9% fed on cattle or goats (Table 6.1 (e)). Thus many of the blood-fed mosquitoes must have entered the huts after feeding and these feeds cannot be "blamed on" failure of the nets. In addition, it is possible that a small

proportion of human blood meals may have occurred outside the hut. Because the results on blood-fed mosquitoes in the 1988 trial were based on the total number of mosquitoes found blood fed (irrespective of blood meal origin), they therefore can give only incomplete evidence on the ability of nets to protect sleepers under them.

6.1.3.6 Chemical analysis of netting.

As shown in Tables 6.1. and 6.1 (c), the amounts of active ingredient on the bednets shown by gas chromatography indicated that there was less than expected for cypermethrin, deltamethrin and lambdacyhalothrin and more than the target for permethrin and pirimiphos-methyl.

The washing regime carried out at the beginning of the trial drastically reduced the levels of a.i., leaving no detectable residues of permethrin or deltamethrin, a 98% reduction of pirimiphos-methyl and 85% reductions of cypermethrin and lambdacyhalothrin.

Over the 12 weeks from initial impregnation to the end of the trial there was a significant loss of cypermethrin, permethrin and pirimiphos-methyl from the unwashed nets (t-tests: $p < 0.05$). Deltamethrin showed no sign of any reduction and the reduction in lambdacyhalothrin was not significant (Table 6.1 (d)).

6.3.1.7 Side effects of sleeping under treated bednets

The survey on the sleepers' health gave 10 reports of ill health out of a possible 216 (4.5%). No correlation between side effect and insecticide was found. Four of the 10 complaints, which included headache and stomach pain, came after nights sleeping under the untreated net (Appendix 6.1 (e)). It is possible that in these cases the sleepers were kept awake by biting mosquitoes and were consequently more aware of any ailments.

Table 6.1

Target and actual deposit densities (mg/m²) as found using G.C. (1988)

<u>Insecticide</u> <u>(Unwashed)</u>	<u>Target</u>	<u>Actual</u>	<u>% Difference</u>
Cypermethrin	100	37.2	-63
Deltamethrin	25	10.2	-59
Lambdacyhalothrin	25	2.6	-90
Permethrin	500	670	+34
Pirimiphos-methyl	1000	4017	+302

TABLE 6.1 (a). Results of Analysis of Variance (ANOVA) for Anopheles gambiae s.l. (1988)

Response analysed	Difference between insecticides			Difference between washed and unwashed nets			Difference in way treated nets changed on washing		
	F	d.f	S.L	F	d.f	S.L	F	d.f	S.L
Total number found in hut	8.3	5,159	***	0.1	1,159	N.S	2.4	5,159	*
Total number dead	21.5	5,159	***	1.5	1,159	N.S	2.0	5,159	N.S
Percentage dead	16.1	5,159	***	11.8	1,159	***	1.3	5,155	N.S
Total number blood-fed	3.6	5,155	**	0.4	1,155	N.S	0.9	5,155	N.S
Percentage blood-fed	2.1	5,155	(*)	0.9	1,155	N.S	0.8	5,155	N.S
Percentage exiting	1.9	5,155	N.S	1.6	1,155	N.S	2.3	5,155	*

S.L = Significance Level :-

(*) $p=0.05$
 * $p<0.05$
 ** $p<0.01$
 *** $p<0.001$

TABLE 6.1 (b). Results of the Analysis of Variance (ANOVA) for Mansonia spp. (1988)

Response analysed	Difference between insecticides			Difference between washed and unwashed nets			Difference in way treated nets changed on washing		
	F	d.f	S.L	F	d.f	S.L	F	d.f	S.L
Total number found in hut	11.8	5,159	***	20.0	1,159	***	9.6	1,159	***
Total number dead	23.4	5,159	***	1.4	1,159	N.S	3.0	5,159	**
Percentage dead	30.5	5,159	***	106.8	1,159	***	7.2	5,159	***
Total number blood-fed	13.8	5,159	***	33.7	1,159	***	11.7	5,159	***
Percentage blood-fed	14.2	5,159	***	11.4	1,159	***	6.8	5,159	***
Percentage exiting	13.3	5,159	***	35.4	1,159	***	2.3	5,159	*

S.L = Significance Level :-

- * $p < 0.05$
- ** $p < 0.01$
- *** $p < 0.001$

TABLE 6.1 (c). Summary statistics of gas chromatography analysis of samples cut from bednets (1988).

Insecticide/ (Target deposit density mg/m ²)	Unwashed Washed U/W	Week	Mean mg/m ²	Standard deviation	Range	% Change week 0-12
Cypermethrin (100)	U	0	37.2	8.3	28-48	
		12	16.0	8.8	7-26	57
	W	0	6.0	1.3	5-8	
		12	1.3	0.5	1-2	78
Deltamethrin (25)	U	0	10.2	7.2	5-24	
		12	10.2	7.9	3-21	0
	W	0	0	0	0	
		12	0	0	0	-
Lambda- cyhalothrin (25)	U	0	2.6	0.9	2-4	
		12	1.6	0.5	1-2	38
	W	0	0.4	0.1	0.3-0.6	
		12	0.5	0.2	0.2-0.7	0
Permethrin (500)	U	0	670	159	380-830	
		12	405	190	170-170	40
	W	0	0	0	0	
		12	0	0	0	0
Pirimiphos- methyl (1000)	U	0	4017	117	3900-4200	
		12	1160	319	800-1600	71
	W	0	10.2	5.5	7-21	
		12	5.3	4.5	2-14	48

TABLE 6.1 (d) Results of t-tests to compare amounts of active ingredient on nets at weeks 0 and 12 (1988)

Insecticide (Unwashed)	t	d.f	p
Cypermethrin	4.3	10	<0.01
Deltamethrin	0	10	>0.10
Lambdacyhalothrin	2.1	10	>0.07
Permethrin	2.6	10	<0.05
Pirimiphos-methyl	20.6	10	<0.001

TABLE 6.1 (e) ELISA Blood meal identification on An.gambiae s.l (1988)

	Human	Bovine	Both H and B	Sheep/ Goat	Unidentified	Total
Number	418	243	20	12	12	705
Percent	59.3	34.4	2.8	1.7	1.7	

Note: 45% of the fed An. gambiae were submitted to blood meal identification;

Key to Figures 6.1.1-6.1.4

Mean number of mosquitoes entering huts per night =

$H + Ws + 2V$ where;

H = number found in room,

W = number found in window traps,

V = number found in verandah traps

N.B. This estimate assumes there are no escape routes other than the window traps and the eaves and no re-entry from the traps to the huts.

In the figures:

U = Untreated

C = Cypermethrin

D = Deltamethrin

L = Lambdacyhalothrin

P = Permethrin

PM = Pirimiphos-methyl

S.E.D = Standard error of the difference between two means.

Key to Figures 6.1.1-6.1.4

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C = Cypermethrin

D = Deltamethrin

L = Lambdacyhalothrin

P = Permethrin

PM = Pirimiphos-methyl

S.E.D = Standard error of the difference between two means.

Figure 6.1.1 Mean number of mosquitoes (x) entering huts per night (1988)

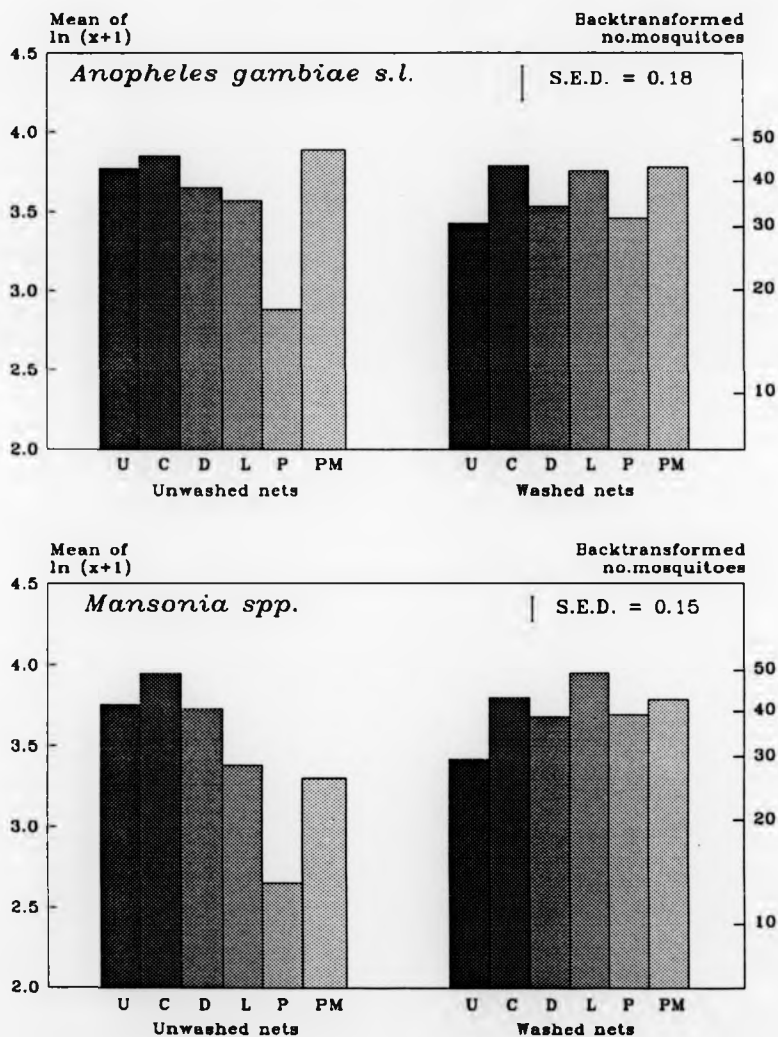
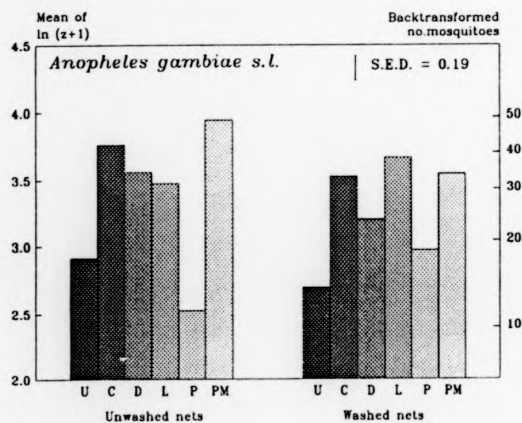


Figure 6.1.2 Mosquito mortality (1988)

(a) Total number killed (z)



(b) Proportion which died (p)

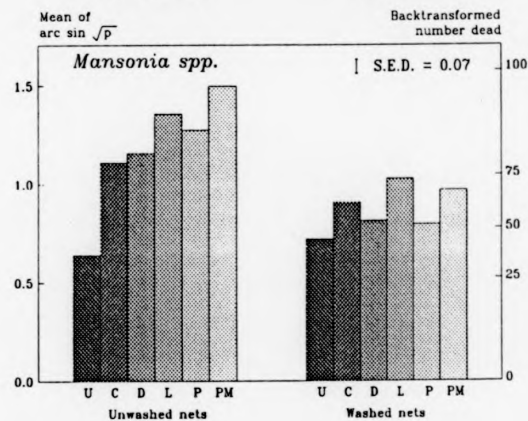
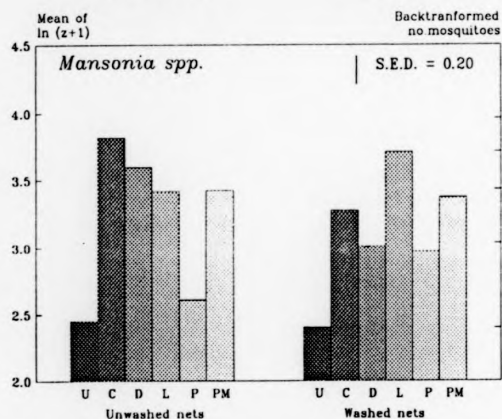
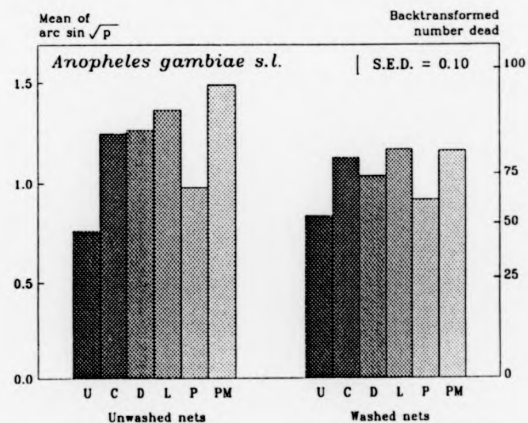


Figure 6.1.3 Mosquito feeding (1988)

(a) Total number (y) found bloodfed

(b) Proportion (q) found bloodfed

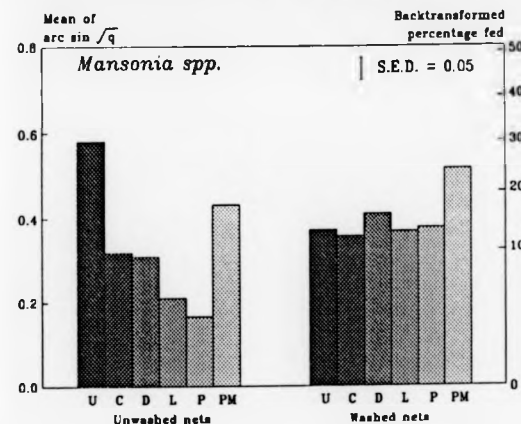
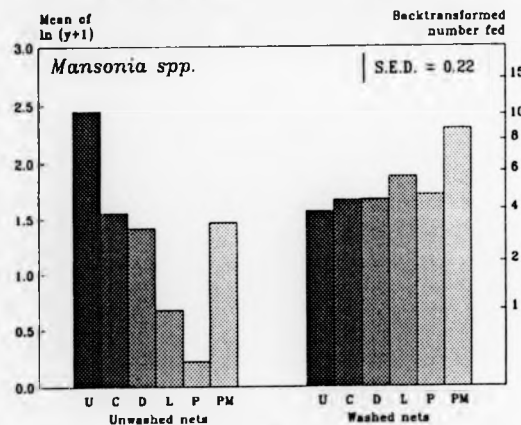
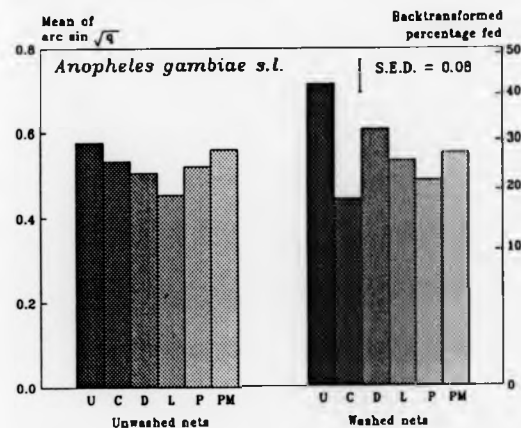
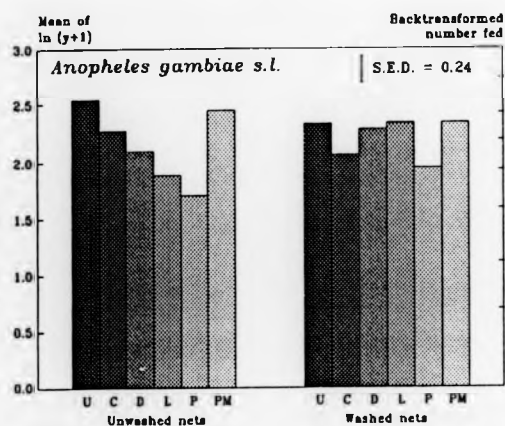


Figure 6.1.3 (c) Percentage of occasions when live bloodfed mosquitoes were found (1988)

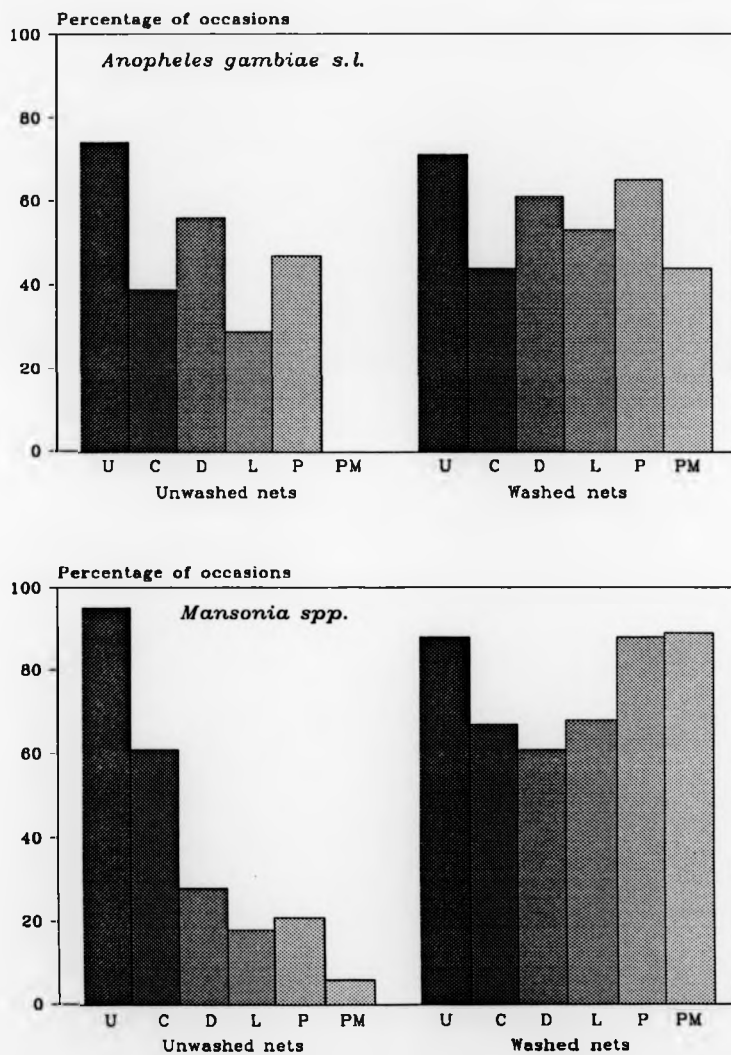
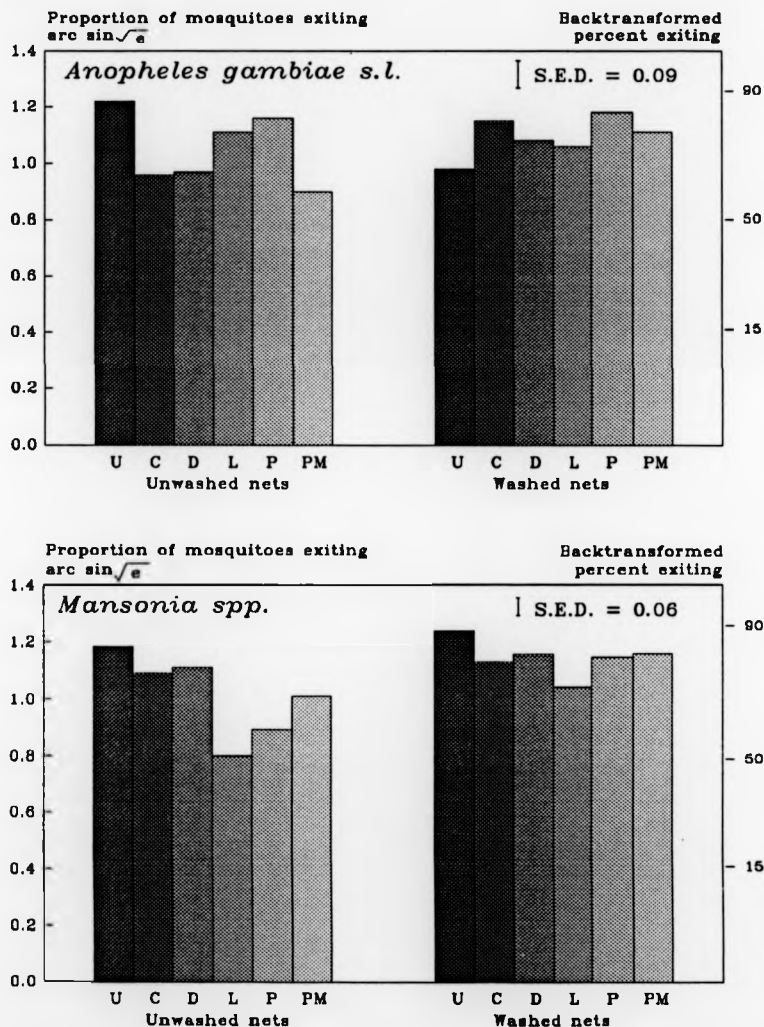


Figure 6.1.4 Exiting (1988):
Proportions of mosquitoes_(e) found in exit
traps and verandahs (*)



6.1.4 DISCUSSION

6.1.4.1 Deterrency

According to de Zulueta and Cullen (1963) the deterrent effect describes "all possible causes that may prevent mosquitoes gaining access to houses treated with residual insecticides". The unwashed permethrin treated net showed a deterrent effect against mosquitoes - the number of An. gambiae s.l. and Mansonia spp. entering huts was estimated to have been 40% or less than that with the untreated net. Darriet *et al.* (1984) reported a similar reduction in the numbers of An. gambiae s.l. and An. funestus entering huts containing 80 mg/m² permethrin a.i. on cotton nets. In this study the unwashed permethrin net was found to have a concentration of 670mg/m² at the start of the trial. Because verandah trap huts were used which can account, (if well built), for all the mosquitoes which enter a hut during the night, the deterrent action of permethrin can be distinguished from an excito-repellent effect which occurs after the mosquitoes have entered the hut and touched the net, or repellency (which might act by vapour action) at short range to drive mosquitoes which had already entered the hut to exit during the night.

The deterrent effect implies action at a distance (i.e. before the mosquitoes pass the eaves and enter the hut) and is difficult to explain, since pyrethroids are considered to have very low vapour pressures. Permethrin has the highest vapour pressure of the pyrethroids used in this trial (1.0×10^{-8} mm Hg at 20°C). Deltamethrin has a vapour pressure of 1.5×10^{-8} mm Hg at 25°C. The vapour pressures of lambda-cyhalothrin and cypermethrin are respectively 1.6×10^{-9} mm Hg and 1.4×10^{-9} mm Hg at 20°C. Pirimiphos-methyl has a much higher vapour pressure than any of the pyrethroids used - 1.5×10^{-5} mm Hg at 20°C. One might have expected far greater deterrency from pirimiphos-methyl due to the higher deposit density used and its higher vapour pressure. However, although the pirimiphos-methyl treated net caused some deterrency of Mansonia spp. it was much lower than that associated with the permethrin net. Thus it seemed likely that some ingredient(s) of

the permethrin formulation was/were acting as a deterrent, and not the active ingredient itself. Recent studies in The Gambia in the same experimental huts confirmed this supposition and have shown that deterrency is caused by chemicals in the blank E.C. and not the permethrin alone (Lindsay *et al.*, in prep). It should also be noted that results of trials of pyrethrum coils in Tanzania showed that the "blank" coils (supposed to be virtually free from pyrethrins and shown by analysis to contain 0.04% pyrethrum) were as deterrent as coils containing six times as much pyrethrum (Hudson and Esozed, 1971).

Deterrency would be a beneficial effect of treated nets for the individual net owner, but mosquitoes could be diverted to unprotected members of the community. Diversion to outdoor animal biting may occur with some species and Lines *et al.* (1987) found that a person sleeping in the same hut as someone protected by a treated net received fewer bites than if no net was used: some degree of protection may be conferred to unprotected people in the immediate vicinity, but it is very doubtful if this would operate between one house and another.

6.1.4.2 Mortality

The total number of mosquitoes killed is an important measure of the effectiveness of the widespread use of nets for community protection. The mean number of days of life in the infective condition is the most crucial factor in the transmission of malaria (Bruce-Chwatt, 1985), and to decrease it is the key to malaria control by the use of adulticides. A high mosquito kill is of benefit to all the community since it confers some degree of protection to those not using bednets, to people before going to bed (Curtis and Lines, 1985; Snow *et al.*, 1988 [b]) and to people who get up before dawn when anophelines are still biting.

The total number of *An. gambiae* s.l. killed by the permethrin treated net was not significantly different from the number of deaths with the untreated net. This was probably because fewer mosquitoes entered the hut when the permethrin net was used

due to its deterrent action and there were thus fewer mosquitoes available to be killed. The unwashed cypermethrin and pirimiphos-methyl nets caused the highest number of mosquito deaths.

However, pirimiphos-methyl has several drawbacks in practical use. The pirimiphos-methyl emulsion was very foamy and it was difficult to wring the nets out which may account for the serious overdosing found using G.C. The nets took longer to dry than the pyrethroid treated nets. They had a pungent odour, felt slightly oily and were the dirtiest nets by the end of the trial. This would be likely to lead to increased washing under domestic conditions.

All insecticide treated nets killed a higher percentage of the An. gambiae s.l. that entered the huts than the untreated net. However, washing decreased the percentages of An. gambiae s.l. killed by the treated nets, and in the case of permethrin, mortality after washing was not significantly different from that with the untreated net. Pirimiphos-methyl was the most insecticidal treatment for Mansonia spp but was not significantly different from lambdacyhalothrin, despite the latter being used at an 800 fold lower concentration. The unwashed lambdacyhalothrin net killed a significantly higher number of the Mansonia spp. which entered the huts than the cypermethrin or deltamethrin nets.

Both killing and deterrence could select for resistance, although the rate at which this would occur may differ. Deterrence might reduce the number killed and therefore reduce selection for resistance genes.

6.1.4.3 Feeding

Prevention of probing and blood-feeding is another very important function of treated nets as it will prevent transmission of malaria and allow a good night's sleep. Without insecticides, mosquitoes can probe through the net or enter holes to feed. Generally mosquitoes do not remain in contact with intact treated nets long enough to probe successfully (Hossain and Curtis, 1989 [b]; section 4.3). However, Snow *et al*

(1987 [a]) found the proportions of bloodfed An. gambiae s.l. were not significantly different in rooms with or without permethrin treated nets. In this study also, the insecticide treated bednets did not decrease the percentages of bloodfed An. gambiae s.l. found in the huts and traps. However, it should be stressed once again, that the analysis of blood feeding for both species of mosquitoes was ^{not} carried out on all mosquitoes found blood fed. The identification of the 705 squashes of An. gambiae s.l. was only carried out to ascertain the proportions human and cattle fed and was not related to net use. It should also be noted that until a procedure such as "DNA fingerprinting" can be used to identify the precise origin of the blood-meal (Coulson et al., in prep) the percentages which actually fed on the occupants of treated and untreated nets, as opposed to those entering the room, after feeding on other humans elsewhere, cannot be established.

Bearing the above in mind and also that no identification of the species origin of blood meals of Mansonia spp. was undertaken, analysis of the fed Mansonia spp. suggested that the pirimiphos-methyl net was the least effective and the permethrin and lambdacyhalothrin nets were the most effective in reducing bloodfeeding. There may be a species difference in susceptibility for physiological or behavioural reasons. Charlwood (1986) states that little attention has been paid to the possibility of a differential response to mosquito bednets by different species. However, his data suggests that nets may be more effective against culicines than anophelines, presumably because anophelines may give up the search for a blood meal more quickly in the face of treated nets than culicines. Generally the percentage of bloodfed individuals was greater for An. gambiae s.l. than Mansonia spp. with all the insecticides. Because of the high proportion of animal fed An. gambiae s.l., these results are difficult to interpret. However, since the unwashed permethrin treated net was associated with the lowest total numbers of bloodfed mosquitoes due to its deterrent effect, it thus provided the best personal protection against vectors of malaria. The total numbers of live bloodfed mosquitoes is proportional to the

probability of onward transmission of malaria. No live blood-fed An. gambiae s.l. were found when the pirimiphos-methyl net was used. Even after washing, the percentage of occasions when live, blood-fed An. gambiae s.l. were found was still lower for all insecticides than for the untreated net. Despite the holes in each net, live bloodfed mosquitoes were never found inside the treated nets (including the washed ones) but were found in the untreated nets.

6.1.4.4 Exiting

This is an important criterion for assessing the possible excito-repellency (or vapour repellency) of the insecticides which may cause mosquitoes to exit before picking up a lethal dose. The percentage of the An.gambiae s.l. caught which were inside the huts (as opposed to in the traps) was significantly lower with the unwashed deltamethrin, cypermethrin and pirimiphos-methyl nets than the untreated net, whereas for Mansonia spp. it was significantly less for the pirimiphos-methyl, permethrin and lambdacyhalothrin nets than for the untreated nets.

After washing the nets, all the insecticide treated nets caused a higher percentage of An.gambiae s.l to exit, which may imply that the reduction in insecticide deposit density was more irritant than the higher pre-wash deposit density which tended to kill before the mosquitoes could escape from the room. Only the washed lambdacyhalothrin net was associated with a significantly lower percentage of Mansonia spp. exiting than was the untreated net.

Smith and Chabeda (1968) found that initially, after spraying with tetramethrin, there was low egress due to a very high proportion of mosquitoes being killed indoors, but, as the insecticide lost its toxicity, its intensely irritant properties were revealed by the high proportion of mosquitoes leaving the treated hut by windows and eaves.

Darriet et al. (1984) in Burkina Faso and Lines et al. (1987) in Tanzania, found that permethrin treated nets caused a much larger percentage of both fed and unfed

mosquitoes (*An.gambiae* s.l. and *An.funestus*) to exit from the huts. The deposit densities used were 80 and 200 mg/m² respectively which may have allowed the mosquitoes to exit before picking up a lethal dose or before becoming knocked down.

The "enforced exophily" which occurs with some treated nets means that a proper assessment of treated nets requires window traps (plus, ideally, verandah traps). If one simply works with numbers found resting inside the rooms of the huts one would get a very incorrect impression of what occurred during the night.

The reason for the underdosing and overdosing of the nets is unclear (Tables 6.1 and 6.1 (c)). Hossain *et al.* (1989 [a]) and Lindsay *et al.* (in press), found good approximations to target dosages of various pyrethroids on netting samples of up to 1/2 sq metre. The problems in the present trial may have been due to the large amount of fabric, making it difficult to wring out the nets evenly. Since the G.C. analyses could not be carried out until after the trial had finished it was not possible to correct for this. According to K.Heales (pers.comm.) other workers, using whole nets, have found wide ranges in deposit densities e.g;

Country	Target mg/m ²	n pieces	Mean mg/m ²	S.D. mg/m ²	Range mg/m ²
Soloman Islands	P 500	6	300	93	200-460
Papua New Guinea	P 500	5	562	169	340-710
Papua New Guinea	P 500	5	932	243	760-1360
Thailand	L 10	10	7.8	4	3-15
Suriname	L 23	1	7.0	-	-
Tanzania	P 200	2	650	14	640-660

P = permethrin; L = lambdacyhalothrin.

When the nets are difficult to wring out because of the type of fibre from which they are made (e.g. the samples from Tanzania which were polyethylene),

deposit densities are usually found to be much higher than intended. Use of a mangle might give a more even distribution of active ingredient.

6.1.5 SUMMARY

- (1) The permethrin treated net afforded the greatest personal protection due to its deterrent activity.
- (2) Permethrin did not kill so many mosquitoes as the other four insecticides and therefore would not be such a good choice for community protection. Lambdacyhalothrin was particularly effective, especially when it is recalled that its deposit density was 0.4% that of the permethrin net and 0.06% that of the pirimiphos-methyl net.
- (3) Washing generally had a detrimental effect on all treated nets: it would be important to impregnate nets just prior to the peak period of malaria transmission in places where malaria is seasonal and brief and when transmission is perennial, one should treat nets as frequently as the local net washing customs dictate, at least until a wash-resistant formulation is available.

6.2 Trial in 1989 of permethrin (P), wash-resistant permethrin (WP), lambdacyhalothrin (L), wash-resistant lambdacyhalothrin (WL) and a mixture of permethrin and pirimiphos-methyl (MIX)

6.2.1 INTRODUCTION

From the hut trial comparing the four pyrethroids and the organophosphate it was concluded that permethrin appeared to be the insecticide of choice for personal protection, whereas lambdacyhalothrin was the better choice for community protection. The efficacy of the insecticides was reduced on washing the nets; three washes, carried out in the traditional manner in cold water and the locally available cow fat soap, left no detectable residues of permethrin and caused an 85% reduction of lambdacyhalothrin.

Incorporation of a polystyrene into the E.C. of permethrin or lambdacyhalothrin was found to reduce the amount of pyrethroid lost on washing treated netting, as assessed by bioassay mortality and G.C. analyses (Chapter 3).

Pyrethroid resistance presents a potential threat to the long term usefulness of pyrethroid impregnated bednets. Theoretically, as previously discussed, a mixture of a pyrethroid with an unrelated compound could delay the build up of resistance.

Thus the aims of the following trial were:-

- (1) to assess, in experimental huts before and after washing, the formulations of permethrin and lambdacyhalothrin incorporating polystyrene,
- (2) to compare the formulations with and without polystyrene of permethrin and lambdacyhalothrin in experimental huts,
- (3) to determine whether the incorporation of polystyrene into the E.C. decreases the deterrent effect of permethrin found in the previous study (6.1) and by other workers,
- (4) to assess the activity of a permethrin and organophosphate mixture.

6.2.2 METHODS AND MATERIALS

The trial was carried out at Walikunda, The Gambia, and was of the same design as that described in the materials and methods section of 6.1.

6.2.2.1 Insecticides

The target deposit densities of each insecticide were:

permethrin - 500mg/m^2 (from 25% E.C., "Imperator", I.C.I.),

wash-resistant permethrin - 500mg/m^2 (from 25% E.C., "Imperator" plus 5% polystyrene, 100,000 m.w., BDH),

lambdacyhalothrin - 10mg/m^2 (from 2.5% E.C., "Icon", I.C.I.)

wash-resistant lambdacyhalothrin - 10mg/m^2 (from 2.5% E.C., "Icon", I.C.I. plus 5% polystyrene 100,000 m.w., BDH)

mixture of permethrin 200mg/m^2 (from 25% "Imperator") and pirimiphos-methyl at 200mg/m^2 (from 50% E.C., "Actellic", I.C.I.).

6.2.2.2 Blood meal Identification

ELISAs (see Appendix 6.1) were carried out on all the An. gambiae s.l. found blood fed ($n=1032$) so that the number fed on human blood in the presence of each net could be compared.

6.2.2.3 Delayed Mortality

Delayed mortality was not measured in An.gambiae s.l. for two reasons. Firstly, the previous trial had shown that negligible mortality occurred during this holding period, i.e. if a mosquito was not dead on arrival at the laboratory, it was unlikely to die in the next 24 hours. Secondly, holding for 24 hours would have meant that the blood meals could not have been accurately analysed.

6.2.2.4 Effect of polystyrene on mosquitoes

To determine whether the polystyrene was affecting mosquito behaviour or mortality, an extra week's trial was carried out. Three placebo nets, X (dipped in water) and 3 treated nets, Y, (dipped in the blank formulation plus 5% polystyrene) were compared. The design is shown in Appendix 6.2 (a).

6.2.2.5 Statistical Analysis

Breakdown of the treatment effect into the three components of treatment, effect of washing on treatment and treatment-by-washing interaction (i.e. how the different treatments changed on washing) and breakdown of the night into three components (6.1.2.11) was not considered necessary. The statistical analyses for bloodfeeding were only carried out on An.gambiae s.l. since bloodmeal identification was only carried out on this species.

6.2.3 RESULTS

The mean actual deposit densities (mg/m^2) on the nets at the start of the trial are shown in Table 6.2.

The following response variables were analysed:

- (i) Total number of mosquitoes collected in the room, window traps plus twice the number in the verandahs of each hut;
- (ii) Mosquito mortality: (a) total number which were found dead, and (b) percentage of the total collection which were found dead;
- (iii) Human bloodfeeding (analysis of Anopheles gambiae s.l. only): (a) total number which were human blood-fed, (b) percentage of the total number found which had fed on human blood-fed, and (c) total number which were found alive and blood fed (i.e. those with the potential for the onward transmission of malaria);

- (iv) Total number of mosquitoes exiting from the hut (number found (live and dead) in window traps plus twice the number in the verandahs divided by total number estimated to have entered the hut (i.e. $H + W + 2V$).

The results of the ANOVA's for An.gambiae s.l. and Mansonia spp. are shown + Appendix 6.2(c) in Tables 6.2. For both mosquito species, the difference between treatments was significant for all the responses analysed, with the exception of percentage of An.gambiae s.l. exiting. Thus t-test comparisons are justifiable.

6.2.3.1 Numbers of mosquitoes collected.

Figure 6.2.1. shows significantly fewer mosquitoes were found (collection in huts + window traps + twice the number in the verandahs) with all the unwashed insecticide-treated nets compared with the unwashed untreated net ($p < 0.01$). Fewest An.gambiae s.l. were found with the mixture (54% fewer mosquitoes than with the untreated net), but this number was not significantly different from the result with any of the other unwashed treated nets. About 46% fewer An.gambiae s.l. entered the hut containing the permethrin treated net than the hut containing the unwashed, untreated net ($t = 3.65$, d.f. = 30, $p < 0.001$). These effects on the numbers collected were lost after washing the nets.

Significantly fewer Mansonia spp. were found in the presence of all the treated nets compared with the untreated net ($p < 0.05$). With the wash-resistant permethrin treated net significantly fewer Mansonia spp. were found (63% compared with the unwashed untreated net) than with the lambdacyhalothrin or wash-resistant lambdacyhalothrin impregnated nets. The permethrin net caused a reduction of 51% in the number of mosquitoes found in the hut compared with the untreated net. After the nets were washed only the wash-resistant permethrin one significantly reduced the number entering the hut ($t = 2.23$, d.f. = 30, $p < 0.05$), compared with the washed untreated net (32% reduction observed).

6.2.3.2 Mosquito mortality

6.2.3.2 (a) Total numbers killed

Figure 6.2.2 (a) shows that all the unwashed and washed treated nets killed a significantly higher number of An.gambiae s.l. than the untreated net ($p<0.001$). For both unwashed and washed nets, those impregnated with lambdacyhalothrin were associated with the highest mosquito mortality - 21 and 18 times more respectively than the untreated net; and the unwashed lambdacyhalothrin net killed 38% more than the unwashed permethrin net.

After washing, the lambdacyhalothrin net killed significantly more than the permethrin one ($t=2.24$, $d.f.=30$, $p<0.001$). The wash-resistant permethrin net killed significantly more than it did without washing ($t=2.17$, $d.f.=30$, $p<0.05$). All the treated nets, both washed and unwashed, killed significantly more Mansonia spp. than the untreated ones ($p<0.0001$). Without washing the lambdacyhalothrin net killed significantly more mosquitoes than the mixture and wash-resistant permethrin nets but, after washing, there was no significant difference between treatments.

6.2.3.2 (b) Percentages killed.

Figure 6.2.2 (b) shows that with all the unwashed insecticide treated nets a significantly higher proportion of the An.gambiae s.l. died than when the untreated net was in use. The lambdacyhalothrin and wash-resistant lambdacyhalothrin treated nets killed a significantly higher proportion than the other three treatments ($p<0.01$).

After washing, all the treated nets, with the exception of the wash-resistant permethrin one, killed a significantly lower percentage of mosquitoes than they did without washing, but this was still significantly more than the washed, untreated net.

The percentage killed by the wash-resistant permethrin net was not changed significantly by washing and, after washing, it killed significantly more than the net with the normal formulation of permethrin ($t=2.07$, $d.f.=30$, $p<0.05$).

All the unwashed treated nets killed a greater percentage of the Mansonia spp. entering the huts than the unwashed, untreated net; the two lambdacyhalothrin treatments killed the most, although this mortality was not significantly different from that with the permethrin net. As with An.gambiae s.l., the unwashed, permethrin net killed a higher percentage than the unwashed wash-resistant permethrin ($t=2.3$, d.f.=30, $p<0.05$). This may have been due to (a) partial inactivation or masking of the permethrin as a result of binding by the polystyrene, and (b) the unintended differences in initial concentrations which were found by Gas Chromatography to be 558 and 390 mg/m² respectively - see Table 6.2 (b).

After washing, all the treated nets killed significantly more than the untreated net and the two wash-resistant formulations killed the highest percentages of Mansonia spp, (61% and 62% WP and WL respectively) which was significantly more than the nets impregnated with the normal formulation of permethrin or the mixture.

6.2.3.3 Human bloodfeeding

ELISA blood meal identification of 1032 An.gambiae s.l. revealed that in 1989, 92% of the bloodmeals had been from humans (Table 6.2 (d)). In the following analyses, only the number found fed on humans with each net were used i.e. the cattle fed meals were deleted from the denominator.

6.2.3.3 (a) Total numbers human bloodfed

As shown in Figure 6.2.3 (a) all the unwashed, treated nets were associated with significantly fewer human bloodfed mosquitoes than the untreated net. After washing, only the two permethrin treatments and the lambdacyhalothrin net were associated with fewer human bloodfed mosquitoes than the untreated net ($p<0.05$). The latter net was the most effective in preventing blood-feeding, with only 19% of the number of human bloodfed found with the untreated net.

6.2.3.3 Percentage human bloodfed.

Figure 6.2.3 (b) shows that with all the unwashed, insecticide treated nets there was a significantly lower percentage of human bloodfed mosquitoes than with the untreated net.

The mixture was associated with the lowest percentage of human bloodfed An.gambiae s.l. and this was just significantly less than with the wash-resistant lambdacyhalothrin net ($t=2.06$, $d.f.=30$, $p<0.05$).

After the nets were washed, only the two permethrin formulations and the wash-resistant lambdacyhalothrin net were associated with a significantly lower percentage of human bloodfed mosquitoes than the untreated net.

6.2.3.3 (c) Live bloodfed mosquitoes

Considering the data as binary (see 6.1) one can obtain a frequency (%) of occasions when live, human blood fed An.gambiae s.l. were found (Figure 6.2.3 (c)).

χ^2 tests to compare the insecticide treated nets with the unwashed/washed, untreated net (Table 6.2 (e)) found that all the unwashed insecticide treatments were associated with significantly fewer occasions when human bloodfed mosquitoes were found, and there was no significant difference between the insecticide treatments.

All the washed treatments were also significantly different from the untreated net, with the exception of the permethrin net.

6.2.3.4 Exiting from the hut

The percentage of mosquitoes which exited is shown in Figure 6.2.4.

For An.gambiae s.l., there were no significant differences between the washed and unwashed nets.

With Mansonia spp., all the unwashed, insecticide- treated nets were associated with significantly smaller percentages of mosquitoes exiting than the

untreated net ($p < 0.001$) and there were no significant differences between the insecticide treatments.

After washing, only the 2 wash-resistant formulations were associated with a significantly smaller percentage of Mansonia spp. exiting than the untreated net ($p < 0.01$).

6.2.3.5 Effect of washing and time in ambient conditions on amounts of

Insecticide remaining on nets

After three washes there were 97.5%, 89% , 96% and 96% reductions in the amounts of permethrin, wash-resistant permethrin and the two lambdacyhalothrin formulations respectively, remaining on the bednets.

Over the 12 weeks from initial impregnation to the end of the trial, there was a significant loss (as shown by t-tests - Table 6.1 (c)) of permethrin from the nets with the normal permethrin formulation ($p < 0.01$) and of pirimiphos-methyl from the net with the mixture ($p < 0.05$).

6.2.3.6 Comparison of blank formulation containing polystyrene and untreated

(water-dipped) nets

When nets treated with polystyrene plus blank emulsion were used, 48% fewer mosquitoes were collected than with the untreated nets. There was no significant difference in the percentage of mosquitoes killed by, or percentage found bloodfed with the polystyrene and blank nets compared with the untreated nets (Appendix 6.2).
(b)

Table 6.2

Target and actual deposit densities (mg/m^2) as found using
G.C. (1989)

Insecticide (unwashed)	Target	Actual	% Difference
P	500	558	+ 10
WP	500	390	- 22
L	10	5.8	- 42
WL	10	10.6	+ 1
P/PM MIX	200	160	- 20

TABLE 6.2 (a). Results of Analysis of Variance (ANOVA) for Anopheles gambiae s.l. and Mansonia spp. (1989)

Response analysed	<u>An. gambiae</u> s.l.			<u>Mansonia</u> spp.		
	Difference between insecticides			Difference between insecticides		
	F	d.f	S.L	F	d.f	S.L
Total number collected	3.9	11,159	***	7.5	11,159	***
Total number dead	19.3	11,159	***	20.8	11,159	***
Percentage dead	28.2	11,159	***	39.4	11,159	***
Total number blood-fed	7.5	11,159	***	N/A		
Percentage blood-fed	5.5	11,159	***	N/A		
Percentage exiting	5.0	11,159	***	21.7	11,159	***

S.L. = Significance Level :-
 *** p<0.001

TABLE 6.2 (b). Summary statistics of gas chromatography analysis of samples cut from bednets (1989)

Insecticide/ (Target conc mg/m ²)	Unwashed Washed U/W	Week	Mean mg/m ²	Standard deviation	Range	% Change week 0-12
Lambdacyhalothrin (25)	U	0	5.8	3.1	3-11	
		12	3.02	1.1	2.1-4.7	48
	W	0	0.31	0.09	0.14-0.37	
		12	0.36	0.03	0.33-0.40	0
Lambdacyhalothrin Wash-resistant (25)	U	0	10.6	5.6	5-21	
		12	7.1	1.5	5-9	33
	W	0	0.39	0.07	0.26-0.46	
		12	0.46	0.08	0.37-0.44	0
Permethrin (500)	U	0	558	142	380-740	
		12	318	78	250-470	43
	W	0	13.7	2.7	12-19	
		12	12.5	1.9	11-16	9
Permethrin Wash-resistant (500)	U	0	390	160	250-680	
		12	282	52	220-340	18
	W	0	42	18	24-65	
		12	49	45	23-130	0
Mixture pirimiphos-methyl/ permethrin 200/200	Perm. U	0	160	154	50-460	
	P.M.	0	152	128	80-400	
	Perm.	12	97	42	58-170	39
	P.M.	12	2.6	3.4	0.8-9.5	98
Mixture pirimiphos-methyl/ permethrin 200/200	Perm. W	0	1.9	0.4	1.6-2.1	
	P.M.	0	0.54	0.09	0.44-0.68	
	Perm.	12	3.0	0.4	2.5-3.6	0
	P.M.	12	0.3	0.04	0.24-0.35	44

TABLE 6.2 (c) Results of t-tests to compare amounts of active ingredient on nets at weeks 0 and 12 (1989)

Insecticide	T	d.f.	P
Lambda-cyhalothrin	2.07	10	>0.05
Wash-resistant L	1.48	10	>0.1
Permethrin	3.63	10	<0.01
Wash-resistant P	1.57	10	>0.1
MIX:			
Permethrin	0.96	10	>0.2
Pirimiphos-methyl	2.86	10	<0.05

Table 6.2 (d) ELISA Blood meal identification on An.gambiae s.l (1989)

	Human	Bovine	Both H and B	Sheep/ Goat	Unidentified	Total
Number	934	45	16	23	16	1032
Percent	90.5	4.3	1.6	2.2	1.6	

Note: all the blood meals of the An. gambiae found were identified.

TABLE 6.2 (e) Percentage of nights when live human bloodfed An.gambiae s.l. were found (1989)

Net	Unwashed #	Washed #
Untreated	17/18 (94%)	16/18 (89%)
L	0/18 (0%) ***	6/18 (33%)***
WL	0/18 (0%) ***	5/18 (28%)***
P	2/18 (11%) ***	12/18 (67%) n.s.
WP	3/18 (17%) ***	7/18 (39%)**
MIX	2/18 (11%) ***	8/18 (44%)**

X^2 tests were used to compare each insecticide treatment with the results for the untreated nets. The significance levels given are for these X^2 tests:

*** $p < 0.001$

Key to Figures 6.2.1-6.2.4

Mean number of mosquitoes entering huts per night =

$H + Ws + 2V$ where;

H = number found in room,

E = number found in window traps,

V = number found in verandah traps

N.B. This estimate assumes there are no escape routes other than the exit traps and the eaves and no re-entry from the traps to the huts.

In the figures:

U = Untreated net

L = Lambdacyhalothrin net

WL = Wash-resistant lambdacyhalothrin net

P = Permethrin net

WP = Wash-resistant permethrin net

Mix = Permethrin + Pirimiphos-methyl net

S.E.D = Standard error of the difference between two means.

Figure 6.2.1 Mean number of mosquitoes (x) entering huts per night (1989)

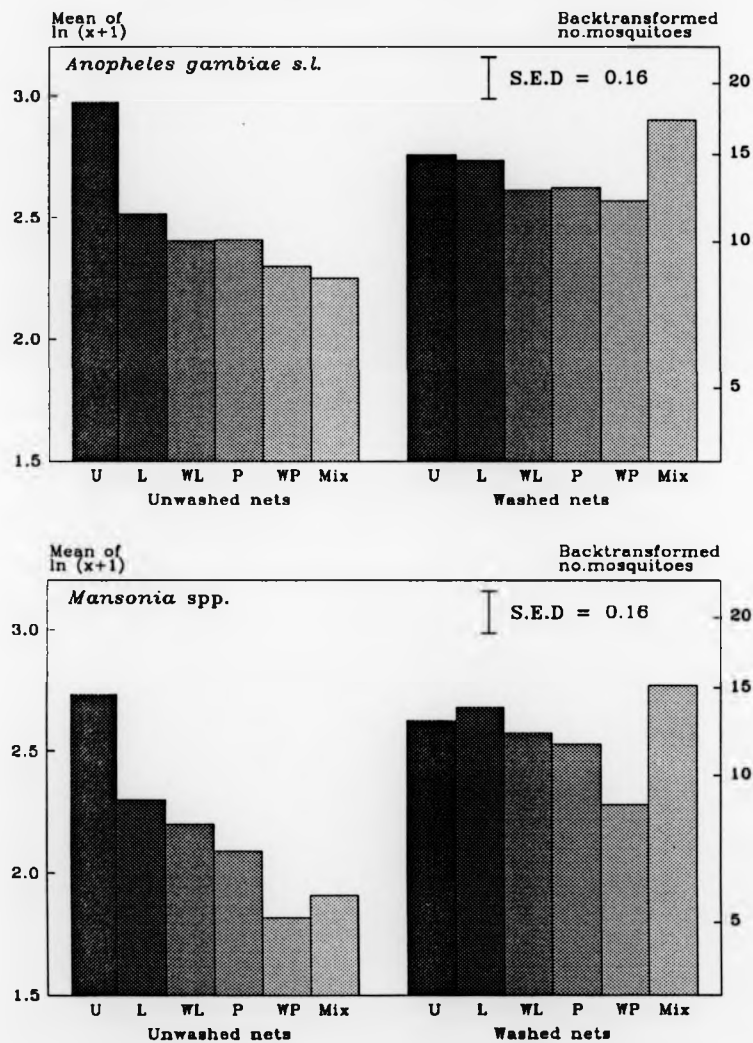


Figure 6.2.2 Mosquito mortality (1989)
 (a) Total number killed (z) (b) Proportion which died (p)

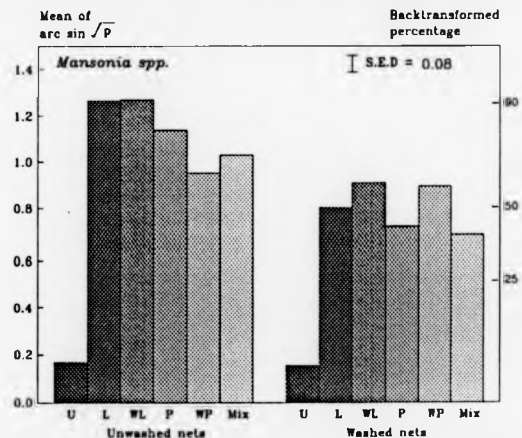
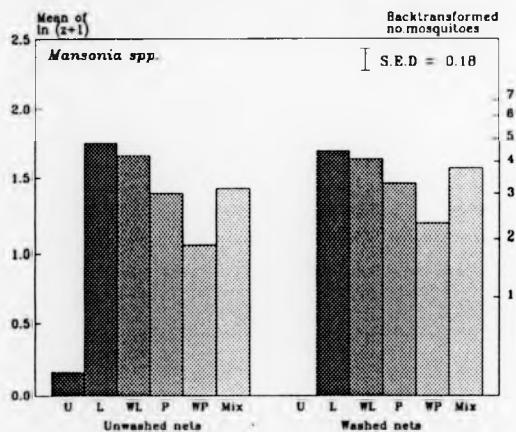
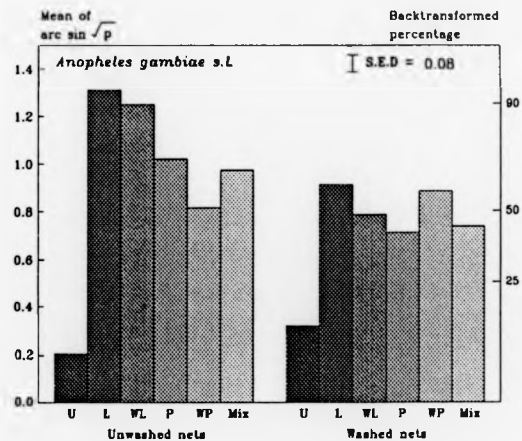
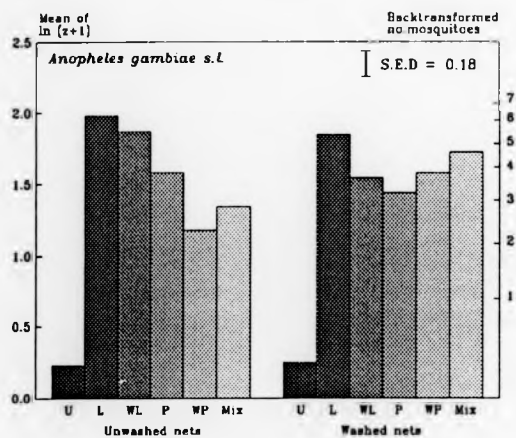
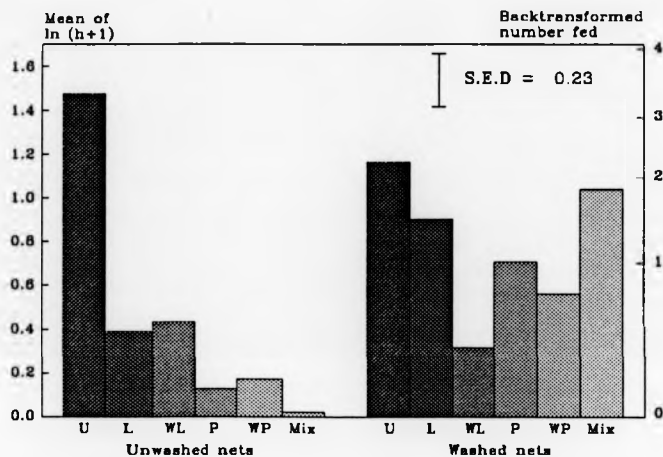


Figure 6.2.3 Human bloodfed *Anopheles gambiae* s.l. (1989)

(a) Total number found with human blood (h)



(b) Percentage (q) of mosquitoes found which contained human blood

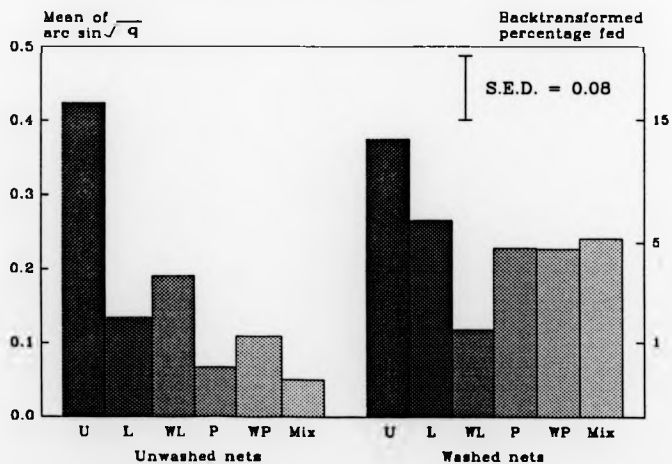


Figure 6.2.3 (c) Percentage of occasions when live bloodfed *An. gambiae* s.l. were found (1989)

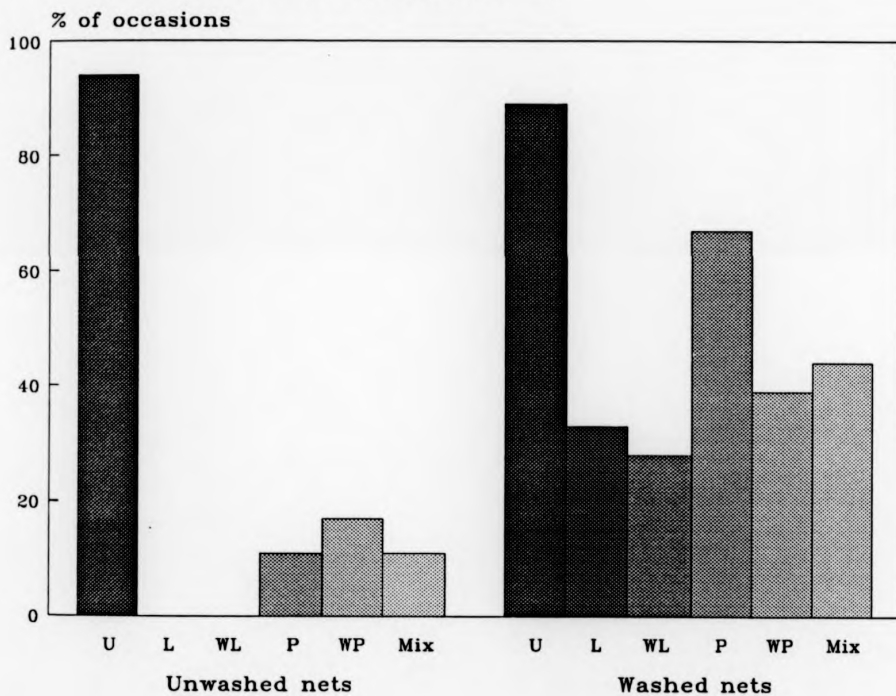
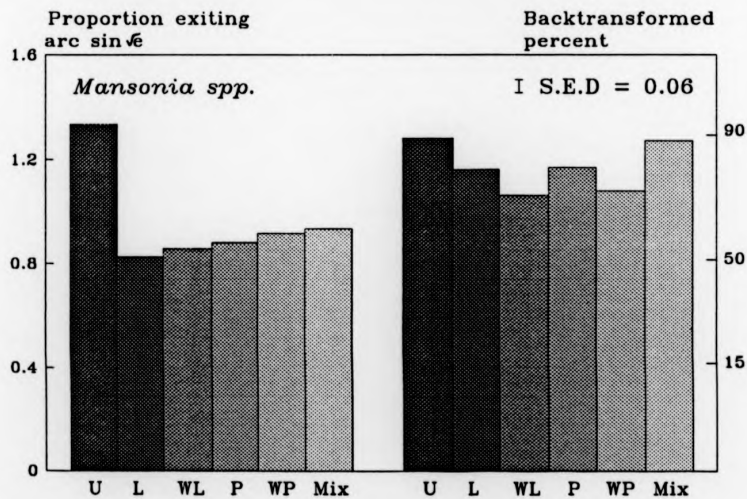
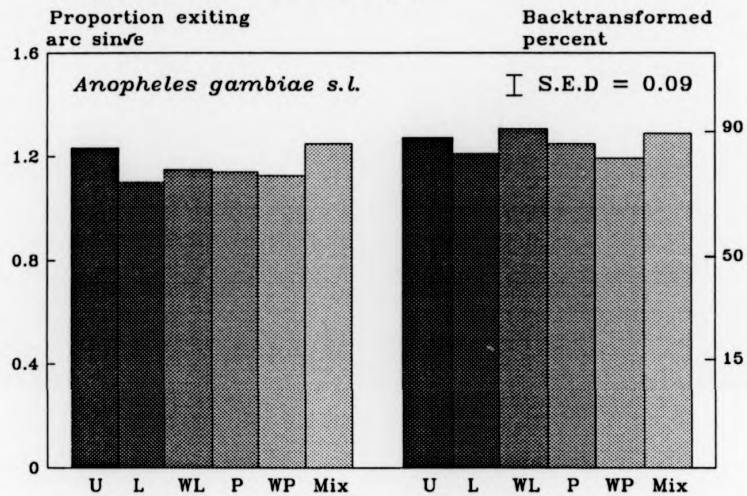


Figure 6.2.4 Exiting (1989):
Proportions of mosquitoes (e) found in exit
traps and verandahs (*)



6.2.4 DISCUSSION

6.2.4.1 Detergency

The total collections in the huts and the traps indicated that all the unwashed insecticide treated nets deterred both species of mosquitoes from entering the huts. There was about a 49% reduction in the numbers of mosquitoes found following use of the permethrin treated net compared to the untreated net. As mentioned in section (6.1.3) the permethrin net in the 1988 trial caused a reduction in the numbers of both species of about 60%. The average deposit densities at the beginning of the 1988 and 1989 trials were 690 and 550 mg/m² respectively.

The unwashed wash-resistant permethrin treated net caused similar detergency to that of the normal unwashed permethrin formulation, i.e. the polystyrene did not prevent the presumed volatilization of the components of the formulation which were acting as deterrents. However, it did not show a significant loss of permethrin over the trial, in contrast to the results with the normal permethrin in both trials.

The unwashed, lambdacyhalothrin treated net had a deterrent effect on both species in the 1989 trial, whereas in 1988 only the Mansonia spp. showed any sign of reduced entry due to this insecticide. In 1989, 39 and 44% fewer An.gambiae s.l. and Mansonia spp. were found respectively, compared to the untreated net. In 1989 the deposit density of the lambdacyhalothrin treated net was twice as much as in the 1988 trial which may have been why it deterred An.gambiae s.l. in 1989.

As already stated, it is likely that it is one, or a combination, of the solvents and emulsifiers in the formulation, and not the pyrethroid itself which is causing the detergency. However, it might be expected that the solvents, which are aromatic hydrocarbons, would evaporate fairly quickly after the nets were dry. The deterrent effect of the permethrin treated net over the 6 weeks of the trial is discussed in 6.3.

The only way to establish which of the components of the E.C. is causing the deterrent effect would be to test them individually. Trials are being carried out in Tanzania (C.Curtis, pers. comm.) to compare the two permethrin formulations most

widely used on bednets - the I.C.I. 25% E.C. "Imperator" and the Wellcome 20% "Peripel" to determine whether, at the same deposit densities, they cause differing levels of detergency.

Although the deterrent effect on An. gambiae s.l. of all the treated nets was lost after washing them, this was not the case for the effect of the wash-resistant permethrin on Mansonia spp. The Mansonia spp. were also more sensitive to the unwashed nets (e.g. numbers collected reduced by 51% by the permethrin treated net, compared to the untreated net). This is another example of a species difference in susceptibility to treated nets which should not be overlooked when planning field trials.

6.2.4.2 Mortality

After washing, all the treatments killed less than they had before washing, but more than the untreated net. In the 1988 trial, the mortality caused by the permethrin net after washing was not significantly different from that of the untreated net. The 1988 G.C. results indicated that there was no permethrin detectable after the 3 washes. However, the 1989 G.C. analysis of the permethrin net indicated that there was about 14 mg/m². One of the women who washed the nets for this trial was different from 1988 and may not have scrubbed so hard as her predecessor.

The important finding was that the percent mortality due to the wash-resistant permethrin did not change significantly after washing and was significantly higher than with the normal permethrin formulation. Furthermore, the wash-resistant permethrin killed a significantly higher number of An.gambiae s.l. after washing than without washing. This implies that the unwashed polystyrene masked the active ingredient to some degree. It is concluded that there is still scope for improvement of the formulation.

6.2.4.3 Bloodfeeding

There were two interesting results on blood feeding in the 1989 trial. Firstly, the ELISA tests revealed that, of the 1032 bloodfed An.gambiae s.l., 90.5% had fed on humans. The 1988 results showed that only 59% of the 705 blood meals analysed (45% of total caught bloodfed) came from man. In 1988, a large herd of cattle was tethered at night a few hundred metres from the huts. It appears that many mosquitoes fed on these cattle and then came into the huts to rest. In 1989, the cattle had been taken to a site more than 5 kms away. This result is an example of the possible use of cattle to reduce mosquitoes feeding on man (zoophylaxis). In some parts of Africa, zoophylaxis is a recognised measure against mosquitoes such as An.arabiensis, i.e. cattle are deliberately impounded near or within houses so that the mosquitoes feed preferentially on the cattle rather than on humans (Burkot,1988). It should be pointed out, however, that the presence of many cattle may increase the bloodfeeding (and hence egg laying) success of the mosquito population and cattle hoofprints are favoured An.gambiae s.l. breeding sites. The pros and cons of zoophylaxis have been considered theoretically by Sota and Mogi (1989).

Secondly, in 1988 it appeared that the insecticide treated nets did not have any effect on the numbers of bloodfed (includes both human and cattle) An.gambiae s.l. (with the exception of live bloodfeds) although the numbers of bloodfed Mansonia spp. were affected (no ELISAs undertaken to distinguish origin of meals). In 1989, all the unwashed untreated nets significantly reduced the numbers bloodfed An.gambiae s.l. bloodfed. The 1989 result is the more meaningful since it reflects the analysis of human bloodfed data only, whereas the 1988 result is based on all mosquitoes found blood fed which includes the 41% which had fed on cattle, sheep and goats. As pointed out in 6.1, these feeds must have occurred before the mosquitoes entered the huts and the nets cannot be "blamed" for failing to prevent such feeds.

6.2.4.4 Exiting

The results of the analysis of the percentages exiting resembled those of the 1988 trial. Interestingly, there again appears to be a species difference in response to the treated nets. The percentages of An.gambiae s.l. found exiting due to the permethrin and lambdacyhalothrin nets were not affected, whereas the percentages of Mansonia spp. were significantly reduced. Other workers (see 6.1) have found that permethrin nets, with lower deposit densities than the ones used in these trials, significantly increased the percentages of An.gambiae s.l. and An.funestus exiting.

6.2.4.5 Mixture

Although the combination of unwashed permethrin and pirimiphos-methyl performed well over the 6 weeks of the trial, as assessed by its effect on mosquito kill and prevention of bloodfeeding, the G.C. results carried out 12 weeks post-impregnation, showed that the amount of pirimiphos-methyl had declined from 152 to 2.6 mg/m². As already discussed, the concept of mixtures to delay resistance requires that the two components decay equally over time so that mosquitoes are exposed to the same degree to both insecticides. Thus, pirimiphos-methyl would not be a suitable choice for one component of a mixture and further studies should be carried out to find a better candidate.

6.2.4.6 Wash-resistant lambdacyhalothrin

This did not perform much better than the ordinary lambdacyhalothrin formulation, except that after washing, the wash-resistant formulation prevented a larger number (and %) of An.gambiae s.l. from bloodfeeding. The E.C. used was 2.5% and it may be that the 5% of polystyrene binds the active ingredient too strongly, although the laboratory tests found decreasing the percentage of polystyrene did not increase the performance of the formulation after washing. The 5% E.C. of

lambdacyhalothrin was not tested, because the 2.5% had been used throughout the studies. However, this formulation should be tested with polystyrene and other polymers in an attempt to improve efficacy of the lambdacyhalothrin formulation.

6.2.4.7 Polystyrene

As expected, the polystyrene on its own did not affect the mortality or bloodfeeding of mosquitoes. The deterrent effect shown by the blank formulation plus polystyrene was due to the other components of the blank formulation (see 6.1).

6.2.5 SUMMARY

- (1) The wash-resistant permethrin performed better than normal permethrin formulation and it performed as well as lambdacyhalothrin in some respects.
- (2) Incorporation of polystyrene into the permethrin formulation did not decrease its deterrent effect.
- (3) The permethrin/pirimiphos-methyl mixture performed well over the 6 weeks of the trial but G.C analysis showed a seriously decayed pirimiphos-methyl deposit density at the end of the trial.

6.3 Further analyses of the results from both trials of impregnated bednets in the experimental huts

6.3.1 INTRODUCTION

A number of questions related to the bed net trials using the experimental huts are addressed in the following section:

- 6.3.1.1. Did the deterrent effect of the unwashed permethrin treated net decrease over the 6 weeks of the trial ?
- 6.3.1.2. Did the mosquito mortality associated with the untreated nets increase over the 6 weeks of the trial due to contamination of the huts ?
- 6.3.1.3.1. Which was the most attractive hut to mosquitoes ?
- 6.3.1.4. Which was the most attractive sleeper to mosquitoes ?
- 6.3.1.5. What effect did rainfall during the day and wind during the day and at night have on the number of mosquitoes found in the huts at night ?
- 6.3.1.6. Did the mosquitoes show a preferred direction for exiting, i.e. to the left or right, and what implications might this have since the verandahs were fixed ?
- 6.3.1.7. What was the recovery rate of dead mosquitoes left out overnight in the huts, i.e. how ant-proof were the huts ?
- 6.3.1.8. What was the collecting efficiency of the two mosquito collectors ?
- 6.3.1.9. Did the unwashed nets used in the 1988 trial cause any appreciable vapour mortality ?

6.3.2 METHODS AND DEFINITIONS

6.3.2.1 Deterrent effect of permethrin net

The deterrent effect was assessed from estimates of the total number of mosquitoes (An.gambiae s.l. and Mansonia spp. analysed separately) which entered the hut. These estimates came from the numbers found in the room, window traps plus twice the number in the verandahs. The estimates were made for each night the unwashed permethrin net was used during the 1989 trials.

To assess possible evaporation or volatilization of the deterrent substances, the numbers of An.gambiae s.l. found during the first 6 nights were compared with the numbers found during the last 6 nights using t-tests.

6.3.2.2 Mosquito mortality with untreated net

The percent mortality of mosquitoes (2 species analysed separately) found using the untreated net over the 36 nights of the trial was assessed using χ^2 tests for trend.

6.3.2.3 Most attractive hut to mosquitoes

To determine the most and least attractive hut the mean number of mosquitoes (An.gambiae s.l. and Mansonia spp. analysed separately) collected per night was calculated for each hut.

6.3.2.4 Most attractive sleeper to mosquitoes

To determine which sleeper was the most and which was the least attractive to mosquitoes, the mean number of each species collected per night in the hut that each sleeper was "on duty" was calculated.

6.3.2.5 Rainfall, wind and mosquito numbers

The number of each species found with the untreated net (1989) was compared with the preceding day's rainfall and the amount of wind during the day (0630-1830) and at night (1830-0630). An anemometer, placed adjacent to, and about 10 m away from hut 3 (Figure 6.1 (a), Plate 4 [a]) was used to record the amount of wind during these time periods. Rainfall data over the period of the trial were obtained from the meteorological office at Sapu approximately 7 km from Walikunda.

6.3.2.6 Numbers in left and right traps

The number of mosquitoes collected from the left and right window traps and the left and right verandahs from each of the 6 huts on 6 mornings were compared. The percent of the total mosquitoes found in the left window trap was compared with the percent found in the right exit trap and likewise for the left verandah and right verandah. The number of times when non zero (i.e. where the percentage exiting was greater than 50%) differences between the left and right verandahs and the left and right window traps were compared using the sign test.

6.3.2.7 Scavenger proofness of huts

Once a week, at 1830 on the Saturday night (the sleepers were not in the huts), 6 mosquito corpses were placed in each of the huts, 2 in the room, 2 in the window traps and 2 in the verandahs. The next morning at 0800 those remaining were collected and counted to assess the numbers removed by scavenging insects etc. in the night.

6.3.2.8 Collecting efficiency

The unwashed nets of the 1988 trial were used at the end of the 6 week trial to assess the collecting efficiency of the 2 mosquito collectors. The same 2 collectors

(JEM + YS) worked throughout the trial and every morning each spent 30 minutes collecting from every hut. After carrying out the usual mosquito search of 60 minutes per hut, each hut was sprayed with "Target" (Reckitt and Colman), a non-residual spray containing d-allethrin (0.3%) and d-phenothrin (0.1%). The open eaves were sprayed first for 3 seconds each and then the verandahs (3 seconds) and the room (6 seconds). The hut was left for 10 minutes and then searched for 15 minutes.

6.3.2.9 Vapour toxicity of unwashed nets

At the end of the 1988 trial the vapour toxicity of the unwashed insecticide treated nets was assessed. Unfed mosquitoes (caught in window traps in the village of Saruja, 6 km from Walikunda) were held in 4 paper cups (approximately 10 mosquitoes per cup). The cups which were partially covered with a pad of cotton wool soaked in glucose, were left overnight (12 hours) in the huts containing the unwashed nets. They had no contact with the nets being held 1 metre away from them.

6.3.3 RESULTS

6.3.3.1 Deterency of permethrin net

The number of mosquitoes found when the unwashed permethrin net was used over the 6 weeks of the 1989 trial is shown in Figure 6.3.1. There was no significant difference (t-tests) in the numbers of *An.gambiae* s.l. found in the first six and last six nights of the trial when the untreated net was used. Thus the density of the wild population did not appear to be changing over the 6 weeks of the trial and the numbers found with the unwashed permethrin net were considered suitable for analysis of a possible change in the nature of the net. The t-test showed a significant difference in the numbers found at the beginning and at the end of the trial ($t = 2.46$, d.f = 10, $p < 0.05$; Appendix 6.3.1). In the last 6 nights of the trial there was no difference in the numbers found with the untreated net compared with the permethrin

net.

Since t-tests showed that there was a significant difference ($t = 2.65$, $d.f = 10$, $p < 0.05$) in the numbers of Mansonia spp. found in the first 6 nights and last 6 nights of the trial when the untreated net was used, further analysis of the numbers found when the permethrin treated net was used, was not carried out (Appendix 6.3.1).

6.3.3.2 Mortality with untreated net

The percent mortality of An.gambiae s.l. and Mansonia spp. over the 36 night of the trial is shown in Figure 6.3.2. There are large fluctuations over the 6 weeks of the trial in the percentage of mosquitoes found dead. χ^2 tests for trend (Appendix 6.3.2) carried out on the numbers of An.gambiae s.l. and Mansonia spp found $\chi^2_{trend} = 13.69$, $d.f. = 1$, $p < 0.001$ and $\chi^2 = 27.93$, $d.f. = 1$, $p < 0.001$, respectively.

6.3.3.3 Most attractive hut to mosquitoes

As shown in Figure 6.3.3., hut 2 was the most attractive to An.gambiae s.l.. This appeared to be true for Mansonia spp. also but the number of mosquitoes found was not significantly different from the number in hut 6. For An.gambiae s.l., huts 3 and 4 recorded the lowest counts, but they were not significantly less attractive than huts 1 and 6. For Mansonia spp., huts 4 and 5 recorded the lowest counts although they were not significantly different from huts 1 and 3.

6.3.3.4 Most attractive sleeper to mosquitoes

As shown in Figure 6.3.4, sleeper C was the most attractive to both mosquito species and was significantly more so ($p < 0.01$) than all other sleepers. The lowest number of mosquitoes of both species were recorded with sleeper A but for An.gambiae s.l. he was not significantly less attractive than sleeper E. For An.gambiae s.l. there was no difference in attractiveness between sleepers B, D and E and

likewise for Mansonia spp. between B,D, E and F.

6.3.3.5 Rainfall, wind and number of mosquitoes

The effect of rainfall on the numbers of mosquitoes is shown in Figure 6.3.5 (a) and the effect of wind is shown in Figure 6.3.5 (b). There does not appear to be a strong correlation between either rainfall or wind on mosquito numbers. (The wind was measured in arbitrary units and since it did not seem to affect mosquito numbers, no further attempt was made to calibrate it).

6.3.3.6 Numbers in left and right traps

The sign test carried out on the exit trap data showed that there was no significant preference for exiting to the left or to the right, and likewise for the verandah trap data (Table 6.3.6).

6.3.3.7 Scavenger-proofness of huts

Table 6.3.7 shows the percentage of dead mosquitoes placed in the huts which were recovered the next morning ranged from 83 to 92%.

6.3.3.8 Collecting Efficiency

As shown in Table 6.3.8, the collecting efficiency ranged from 81 to 92%.

6.3.3.9 Vapour toxicity of nets

Table 6.3.9 shows that in the test for vapour toxicity only the unwashed pirimiphos-methyl net caused mosquito mortality (81%) at a level which was significantly different from the untreated net.

Figure 6.3.1 (a) Number of mosquitoes found when the unwashed permethrin net was used during the 1989 trial

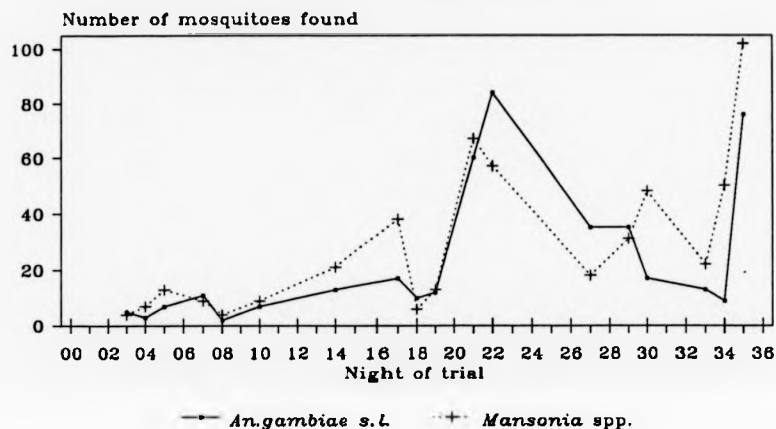


Figure 6.3.1 (b) Number of mosquitoes found when the untreated net was used during the 1989 trial.

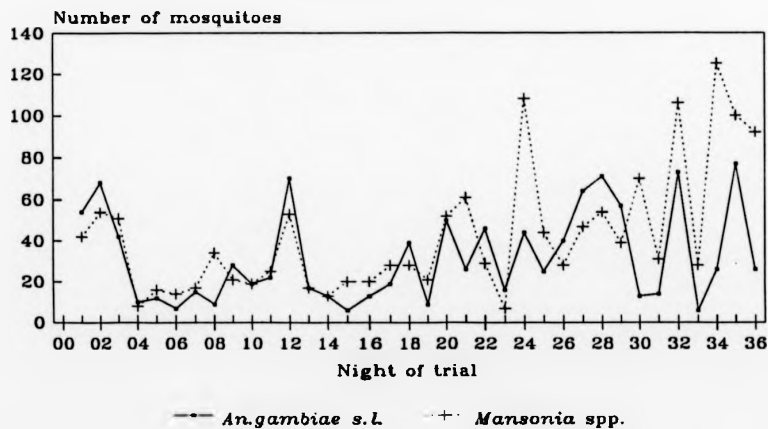


Figure 6.3.2 Percent mortality of *An. gambiae* s.l. and *Mansonia* spp. when the untreated nets were used in the 1989 trial

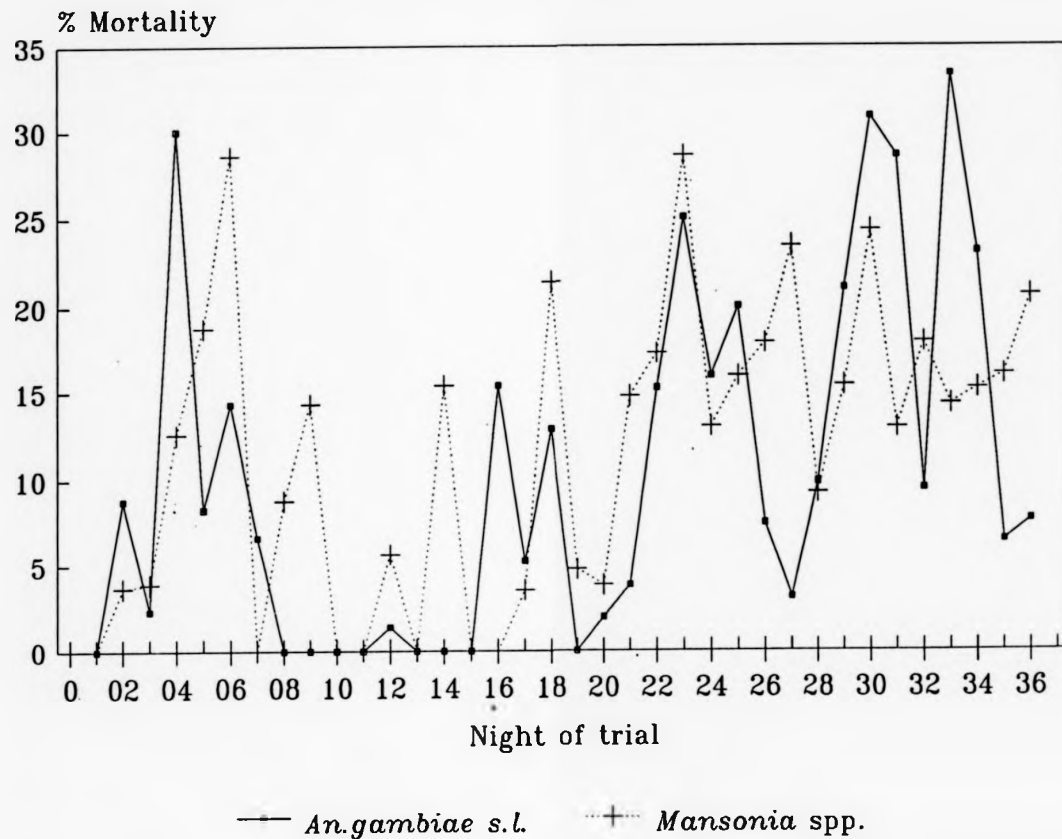


Figure 6.3.3 Number of mosquitoes (n) estimated to have entered each hut per night

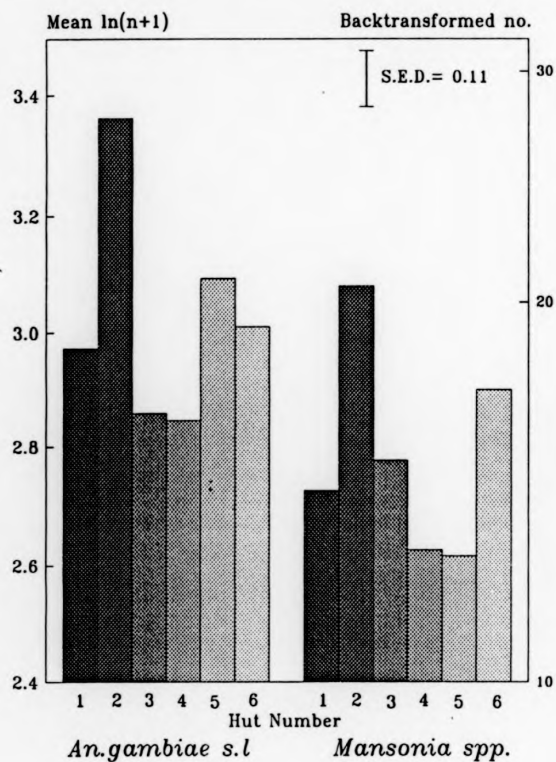


Figure 6.3.4 Number of mosquitoes (n) estimated to have entered huts occupied by each sleeper

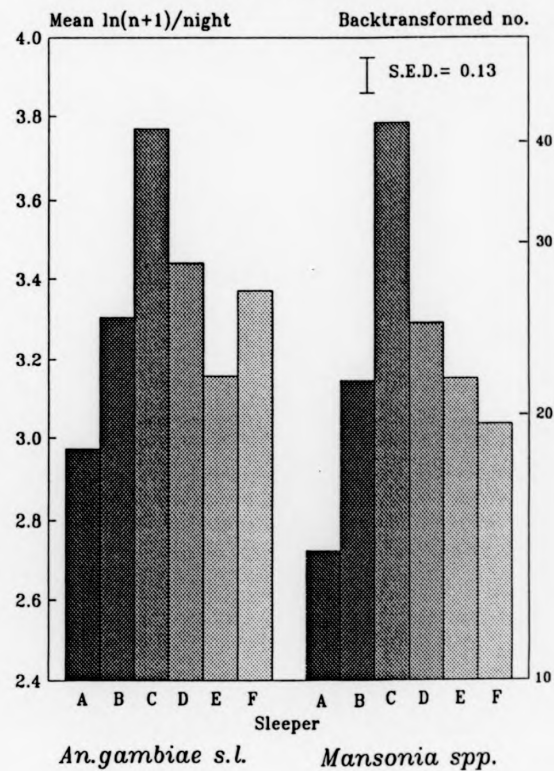


Figure 6.3.5 (a) The effect of rainfall on numbers of mosquitoes found when the untreated net was used.

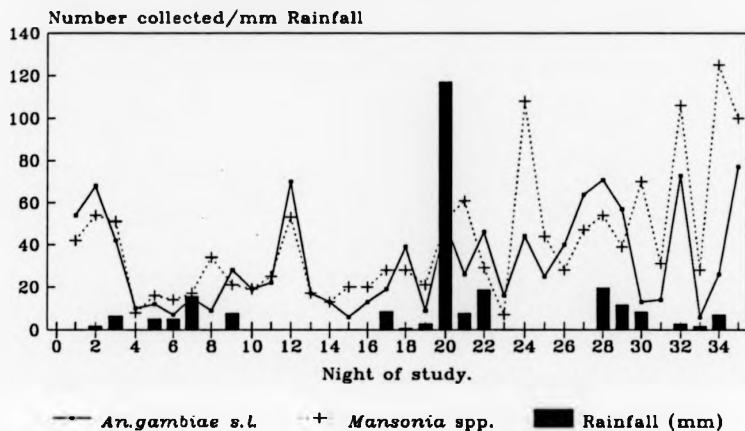


Figure 6.3.5 (b). Effect of wind during the day and at night on the number of mosquitoes found.

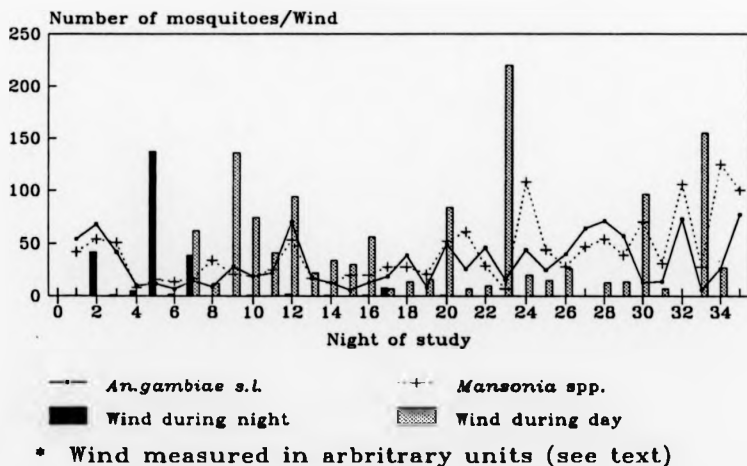


TABLE 6.3.6

Sign test to determine whether mosquitoes showed a directional preference for exiting from huts

Test of null hypothesis that mosquitoes have an equal probability of going right or left.

N = number of non-zero differences between the right and left.

T+ = number of positive differences where positive indicates more mosquitoes exited to the right.

T- = number of negative differences where negative indicates more mosquitoes exited to the left.

T = smaller of T+ and T-

Verandahs

N = 23

T+ = 8

T- = 15

Average rank (A.R.) = 12

Sum ranks for T+ = $8 \times 12 = 96$

Sum ranks for T- = $15 \times 12 = 180$

.. T = 96

N = 23

From Tables* ; $p > 0.1$: Null hypothesis
not rejected

Window traps

N = 34

T+ = 13

T- = 21

A R = 17.5

$13 \times 17.5 = 227.5$

$21 \times 17.5 = 367.5$

.. T = 227.5

N = 34

$p > 0.1$: Null hypothesis
not rejected

* Kirkwood, B. (1988), The Essentials of Medical Statistics

TABLE 6.3.7 Recovery of mosquito corpses left to assess the presence of scavenging insects in the huts

Week	Number of corpses remaining/ Total left out	Percent recovery
1	32/36	89
2	30/36	83
3	33/36	92
4	33/36	92
5	31/36	86

TABLE 6.3.8 Collecting efficiency

Net (washed)	Number caught before pyrethroid spraying (B)	Number collected after spraying (A)	Collecting efficiency (%) $100[(B)/(A)+(B)]$
Untreated	100	8	93
Deltamethrin	63	5	93
Cypermethrin	46	6	88
Lambdacyhalothrin	29	7	81
Permethrin	22	5	81
Pirimiphos-methyl	107	18	86

mean = $87\% \pm 5.4$
± s.d.

TABLE 6.3.9 Mortality of mosquitoes after 24 hours in room containing net but not in contact with net.

Net	Number dead	Total number	% Mortality
Untreated	1	41	2
Deltamethrin	0	41	0
Cypermethrin	2	40	5
Lambdacyhalothrin	3	41	7
Permethrin	0	40	0
Pirimiphos-methyl	30	37	81

6.3.4 DISCUSSION

As explained in section 6.1, the number found in the huts and window traps plus twice the number in the verandah traps is assumed to be a measure of the numbers which entered the hut. In reality, the accuracy of this estimate would depend on the state of repair of the huts (especially the roof) and the degree to which ants and other predators are prevented from entering and removing the corpses of mosquitoes. The huts in Walikunda were completed in July 1988, shortly before the first trial began and so were in excellent condition for that trial at least. Ants were rarely seen and recovery of mosquito corpses deliberately left overnight was very high (approximately 88%). However, it was impossible to keep out lizards and geckos and on one occasion a bat was found in an exit trap. On two other occasions a bird was found in one of the verandahs traps.

Smith and Hudson (1972) point out that the number which entered the hut would be underestimated if mosquitoes which had gone out on to the verandahs, returned to the hut and exited through the open eaves. They would thus have another chance of finding the two open eave gaps, and the assumption that half of those which left the hut would be trapped in the verandahs would be invalid. An excito-repellent treatment might stimulate abnormal flight activity back and forth through the eaves and the repellent action might be wrongly interpreted as deterrence.

I do not consider this a likely source of error for the following reason. The studies reported in Chapters 4 and 5 indicate that mosquitoes quickly become knocked down after only a few minutes contact with netting treated with permethrin or lambda-cyhalothrin at the deposit densities used in these hut trials. Even before knock down, flight is very uncoordinated, and it is difficult to believe that "intoxicated" mosquitoes would easily be able to find the 10 cm wide open eave.

However, to further investigate this, one could attempt to make it more difficult for mosquitoes to egress through the open eaves, whilst not hindering their entry into the huts. Service (1976), quotes Smith and Hudson (1972) who used a wire

baffle fitted to the inside of the hut wall and found it greatly reduced the numbers of An.gambiae s.l. escaping through the eaves. One could also fit a similar baffle to the eaves enclosed by the verandahs so that mosquitoes would find it much more difficult to get back into the room once they had egressed. To test the correctness of the estimate of the numbers entering, mosquitoes marked with fluorescent dust should be released into the huts. Collections of marked mosquitoes in the morning and application of the usual ($H + W + 2V$) formula to the results should account for all the mosquitoes released if the estimation method is correct.

Mosquito mortality associated with the untreated nets increased over the 6 weeks of the trial. This was examined because it was felt that the treated nets, especially the pirimiphos-methyl one, might contaminate the huts. Contamination of huts has occurred in recent studies in Tanzania with lambdacyhalothrin nets (T.J.Wilkes, pers. comm.). Thorough cleaning and airing of huts and washing of exit traps should be carried out between trials. The effectiveness of the decontamination should be monitored by assessing the mortality of mosquitoes held in cups in the huts over night.

The verandahs and exit traps were provided with glucose on cotton wool pads in an attempt to prevent mosquitoes from dying of starvation. On only one occasion was An.gambiae s.l. seen on one of these, although Mansonia spp. were often seen.

The ANOVA's showed there was usually significant heterogeneity between huts and between sleepers (e.g. Appendix 6.1 (b)). Further analysis of this revealed that there was also differences between the mosquito species.

Hut number 2 was the most attractive to both mosquito species, although its attractiveness did not differ significantly from hut 6 for Mansonia spp. (Figure 6.3.3). The positions of the huts are shown in Figure 6.1 (a) and Plate 4 [a]. There does not seem to be any reason why this hut should be more attractive to mosquitoes. The huts were all built at the same time by the same people using similar construction materials. Thus, it is not surprising that one finds such great variations in the numbers of mosquitoes found in houses in villages, given the even greater number of variables

in those situations.

As pointed out by Bidlingmayer and Hem (1980) "trap site bias" is a term used to recognise the existence of poorly understood factors held responsible for the large differences which occur between mosquito catches in traps at apparently similar locations. Their study indicates that the physical features of the terrain that may serve as visual attractants and even slight air currents are the two major factors affecting the numbers of mosquitoes caught.

One of the sleepers was significantly more attractive than all the others for both species (Figure 6.3.4). That individuals vary in their attractiveness to mosquitoes has been known for a long time. Differential mosquito attack rates have been attributed to, amongst other things, host race, age or size, health and use of bednets, host defensive behaviour and proximity of hosts to breeding sites (Burkot, 1988). In this trial, the men were aged between 30 and 45. It would be interesting to analyse the amount of CO₂ expired and body odours of these men to determine why one of them was so much more attractive to mosquitoes than the others. G.C. analysis of the body emanations of the all men might reveal the unknown ingredient.

Rainfall and wind did not seem to have any obvious effect on the numbers of mosquitoes found (Figures 6.3.5 (a) and (b)). The numbers found with the untreated net were used in this analysis because of the deterrency of the other treatments. However, it is interesting to note in Figures 6.3.5 (a) and (b) and Figure 6.3.1 (b) how the numbers of each species rise and fall together. Generally, fewer An.gambiae s.l. than Mansonia spp. were found.

There did not seem to be any preferred direction for exiting by the mosquitoes (Table 6.3.6). In the original concept of Smith (1965) the positions of the verandah traps should be alternated from N-S to E-W because of the possibility that mosquitoes might exit selectively e.g. towards the rising sun, and because any such bias would invalidate the $(H + Ws + 2V)$ formula for estimating the number of mosquitoes entering the hut. However, the lack of bias found between the members of the pairs of

verandah traps at Walikunda retrospectively justified the lack of the capacity to vary the verandah positions.

The collecting efficiency of the mosquito collectors was found to be high, with a mean of about 87% recovery of mosquitoes in the huts (Table 6.3.8). Most mosquitoes, particularly those bloodfed were found around the bottom of the walls inside the room and fairly low down in the verandahs. Gentle tapping of the thatch tended to reveal mosquitoes resting in the roof.

The unwashed pirimiphos-methyl net (which G.C. analysis found had a deposit density of about 1000 mg/m² at the end of the trial (Table 6.1 (c)) caused 81% mortality of mosquitoes held in cups in the 4 corners of the room. This sort of insecticide might cause high mortality due to sublimation on the walls of huts.

6.3.5 SUMMARY

- (1) Deterrency of An.gambiae s.l. (i.e. reduction in the numbers of mosquitoes entering huts) when the permethrin net was used decreased over the 6 weeks of the trial.
- (2) The mortality associated with the control net increased over the 6 weeks of the trial.
- (3) Both sleepers and huts differed in their attractiveness to mosquitoes.
- (4) Neither rainfall nor wind seemed to have an effect on the numbers of mosquitoes found with the untreated net.
- (5) Mosquitoes did not show a preferred direction of exiting from the huts between the two directions which were covered by verandah and window traps.

- (6) Recovery of dead mosquitoes deliberately left in the huts was 88% and collection efficiency of live mosquitoes was 87% so the counts of live and dead wild mosquitoes are considered to have been fairly accurate.
- (7) The vapour toxicity caused by the unwashed pirimiphos-methyl net was 81% after 12 hours.

CHAPTER 7.0

VILLAGE TRIAL OF BEDNETS IMPREGNATED WITH WASH-RESISTANT FORMULATION OF PERMETHRIN OR WITH NORMAL FORMULATIONS OF PERMETHRIN OR LAMBDA CYHALOTHRIN FOR VECTOR CONTROL

7.1 INTRODUCTION

Recent studies have demonstrated that pyrethroids used for bednet treatment tend to be lost from the fabric when bednets are washed (1.14, Chapters 3 and 6). The experimental hut trials (Chapter 6) gave information on the responses of *Anopheles gambiae* s.l. and *Mansonia* spp to different treatments on bednets which had been washed three times or were unwashed. Chapter 3 reports on the loss of active ingredient of normal formulations after one and two washes and on the development of a wash-resistant formulation of permethrin by the addition of polystyrene to the E.C.

A trial was carried out in a Gambian village, simultaneously with the 1989 hut trial, to evaluate the relative entomological impact and persistence of wash-resistant permethrin treatment of bednets in normal use, subjected to regular washing, and compare these with normal formulations of permethrin and lambda cyhalothrin and placebo treated nets. In addition the acceptability of lambda cyhalothrin was monitored, since this pyrethroid has recently been shown to cause some side-effects in village trials in Tanzania (Lines *et al* in prep).

7.2 METHODS AND MATERIALS

The trial was carried out in a large, predominantly Mandinka, village called Saruja (Figure 7 and Figure 6.1 (b)) on the South bank of the River Gambia approximately 300 kms from the coast. In this village almost everyone slept under bednets made of various types of material. The trial was carried out with these nets. At the village meeting, at which permission was sought for the trial, it was explained

that a comparison of 4 chemicals was to occur in order to determine which one killed mosquitoes the best and which one worked the best after the nets were washed.

7.1.1 Impregnation of bednets

One net from each compound was randomly chosen. It was impregnated with one of the following four treatments:

Permethrin (25% E.C. "Imperator", I.C.I.) 500mg/m² normal E.C. formulation [P]; permethrin shown in laboratory tests to be a wash-resistant formulation (25% E.C. "Imperator", I.C.I. + 5% 100,000 m.w. polystyrene, BDH) 500 mg/m²[WP]; lambda-cyhalothrin (2.5% E.C. "Icon", I.C.I.) 25mg/m² E.C. formulation [L] and placebo (milk), at a deposit density of "500"mg/m² [PL].

The treatments were allocated to the compounds using random number tables. One hundred and thirty compounds (98% of the village compounds) took part in the trial. The amounts of emulsion used to impregnate the different types of bednet material were based on those described by Snow *et al* (1987 [a]) and pilot studies carried out in Saruja. The concentration of emulsion was adjusted to compensate for the different uptake of liquid of the different netting types. The impregnation was carried out by the author and 3 field workers. Each net was dipped separately in a large plastic bowl. It was left to dry outside but out of direct sunlight. When it was dry, each net was numbered with the number of the compound using a permanent marker so that it was easily recognizable. It was also marked with a water soluble pen so that if a net was washed this could be recognized.

The chosen bednets were treated in mid-July and evaluated through the following rainy season on a weekly basis for 16 weeks until mosquito populations fell below workable levels in the ensuing dry season. The net users were asked to leave their nets down and tucked in after they got up on Sunday mornings. The women were advised to wash their nets according to their normal routine.

7.2.2 Bednet Searches

One morning a week (Sunday) between 0700 and 1000 hours, each of the treated bednets was searched for mosquitoes using a torch and an aspirator. Any mosquitoes caught were scored as fed or unfed and kept for identification. The net was checked to see if it had been washed, i.e if the water soluble mark was gone. If this was the case, another mark was made. The searchers, with the exception of the author, did not know the allocation of the treatments, to reduce the risk of bias.

7.2.3 Bioassays

Six nets were randomly selected from each treatment group. A WHO plastic cone was placed on the net and the area it covered was marked using a permanent pen. This enabled the same piece of material to be bioassayed weekly. The cone was held in position manually (so that there was no possibility of removing insecticide by the use of sticky tape, etc) and up to 10 mosquitoes were gently introduced into it using an aspirator. The mosquitoes were of wild origin and unfed. They were caught in exit traps in two villages more than 2 kilometres from Saruja. A hand placed behind the cone acted as an attractant to the mosquitoes, helping to keep them in contact with the net (Plate 6). After a three minute exposure time the mosquitoes were removed and placed in paper cups with a pad of 10% glucose solution on the gauze top. Knock-down was scored after one hour and mortality after 24 hours.

7.2.4 Questionnaires

Questionnaire no.1 on net use, net washing, fabric type etc. (Appendix 7.1) was given to every available person in the village. Where the children were too young to reply, the type of fabric and condition of the net only was recorded (see questionnaire). Questionnaire no.2 concerned why people used a net and what they

liked or did not like about treated nets (Appendix 7.2).

7.2.5 Chemical analyses of treated fabrics

In order to assess pyrethroid uptake, 1 m² samples of each of the three main fabric types used in the village were impregnated with each of the pyrethroid treatments. Samples of these three fabrics were sent to I.C.I. Fibres Division for analysis. Three pieces, measuring 10 x 10 cms, were analysed for pyrethroid deposit density using gas liquid chromatography.

7.3 RESULTS

7.3.1 Bednet searches

Table 7.1 shows the total number of mosquitoes found each week in all the nets which had been given each treatment. The average number of nets searched per treatment per week was 30. The percentage of placebo treated nets containing mosquitoes each week varied from 22% (week 14) to 59% (week 4) and the average was 39.6% (s.d. = 8.9%). 89% of the total number of mosquitoes caught under the placebo treated nets were blood fed.

After 16 weeks there was no marked difference in the number of mosquitoes found in the three pyrethroid treatment nets and all three had far fewer than the placebo net group.

Identification of 603 mosquitoes caught in the placebo treated nets revealed 359 An. gambiae s.l. (59.5%), 237 Mansonia spp.(39.3%) and 7 An. pharoensis and An. ziemanini (1.2%).

7.3.2 Bioassays

The bioassay data from each of the 6 nets in the four treatments was divided into those on nets which had received 0, 1, 2, and 3 washes since impregnation

(regardless of the time after impregnation at which the test was done). The placebo mortality for each week was pooled and in the weeks when it exceeded 20% (weeks 6 and 13) the bioassay results for the pyrethroid treated nets were not included in the analysis. For the other weeks, the proportions of mosquitoes killed in each of the washing groups (i.e., 0, 1, 2 and 3 washes) in the three pyrethroid treatment groups were then corrected for the corresponding week's placebo mortality using Abbott's formula and arc sin \sqrt{p} transformed to stabilize the variance. A graph of the back-transformed mean mortalities \pm 95% confidence limits was drawn against number of washes for each pyrethroid treatment (Figure 7.1). The normal permethrin treatment caused the lowest mortality after 3 washes, significantly less than the wash-resistant permethrin, although the 95% confidence limits just overlapped with the lambdacyhalothrin treatment. The wash-resistant permethrin formulation appeared to be the least affected by washing, although the 95% confidence limits overlapped with those of lambdacyhalothrin. Regression analyses of mortality on number of washes are shown in Table 7.2. For the permethrin and lambdacyhalothrin treatments, the linear regressions of mortality on number of washes were very highly significant. For the wash-resistant permethrin, the corresponding linear regression was not significant. Figure 7.2 shows the 95% confidence limits for the regression coefficients of mortality on number of washes. The confidence limits of the regression coefficient of the wash-resistant permethrin do not overlap with those of the permethrin and lambdacyhalothrin, which overlap with each other. It may be concluded that the effect of washing on bioassay mortality of the wash-resistant permethrin was significantly less than the other two treatments which did not differ significantly from each other.

Table 7.4 shows the number of washes received by the nets in each treatment group up to week 16. Only one net had been washed more than 5 times. Figure 7.3 shows the mean (weighted) number of washes that nets in each treatment group had received at weeks 4, 8, 12 and 16 of the trial. Chi-squared tests on number of washes each treatment had received at week 16 showed that there were significant differences

between the lambdacyhalothrin and the permethrin and the lambdacyhalothrin and placebo treated nets ($\chi^2 = 3.95$ (with continuity correction), d.f. = 1, $P < 0.05$; and $\chi^2 = 5.9$ (with continuity correction), d.f. = 1, $P < 0.05$, respectively).

7.3.3 G.C. analyses

The results of the G.C analyses are given in Tables 7.4 (a) and (b). The mean (\pm standard deviation) deposit densities found on permethrin, wash-resistant permethrin and lambdacyhalothrin treated fabrics were 457 ± 163 , 493 ± 138 and 21 ± 8 mg/m² respectively.

The results of the fibre analyses were: (1) the synthetic netting was polyester, approximately 40g/m²; (2) the synthetic sheeting was polyester, approximately 60g/m²; (3) the heavy synthetic sheeting was polyamide (nylon), approximately 245g/m². As already stated in the methods section when the impregnation was undertaken, the concentration of the emulsion was adjusted to compensate for the different uptake of liquid by the different types of fabric.

7.3.4 Questionnaires

In the survey (Questionnaire no.1) carried out on bednet use, washing practices etc, 1328 people were interviewed (an estimated 91% of total population of the village of Saruja). The results are presented in Tables 7.5 (a) and (b). The results of a questionnaire (no.2) on what people liked and disliked about the treated bednets are given in Table 7.6.

Figure 7 MAP OF SARUJA (1989) after Lindsay et al. (in prep)

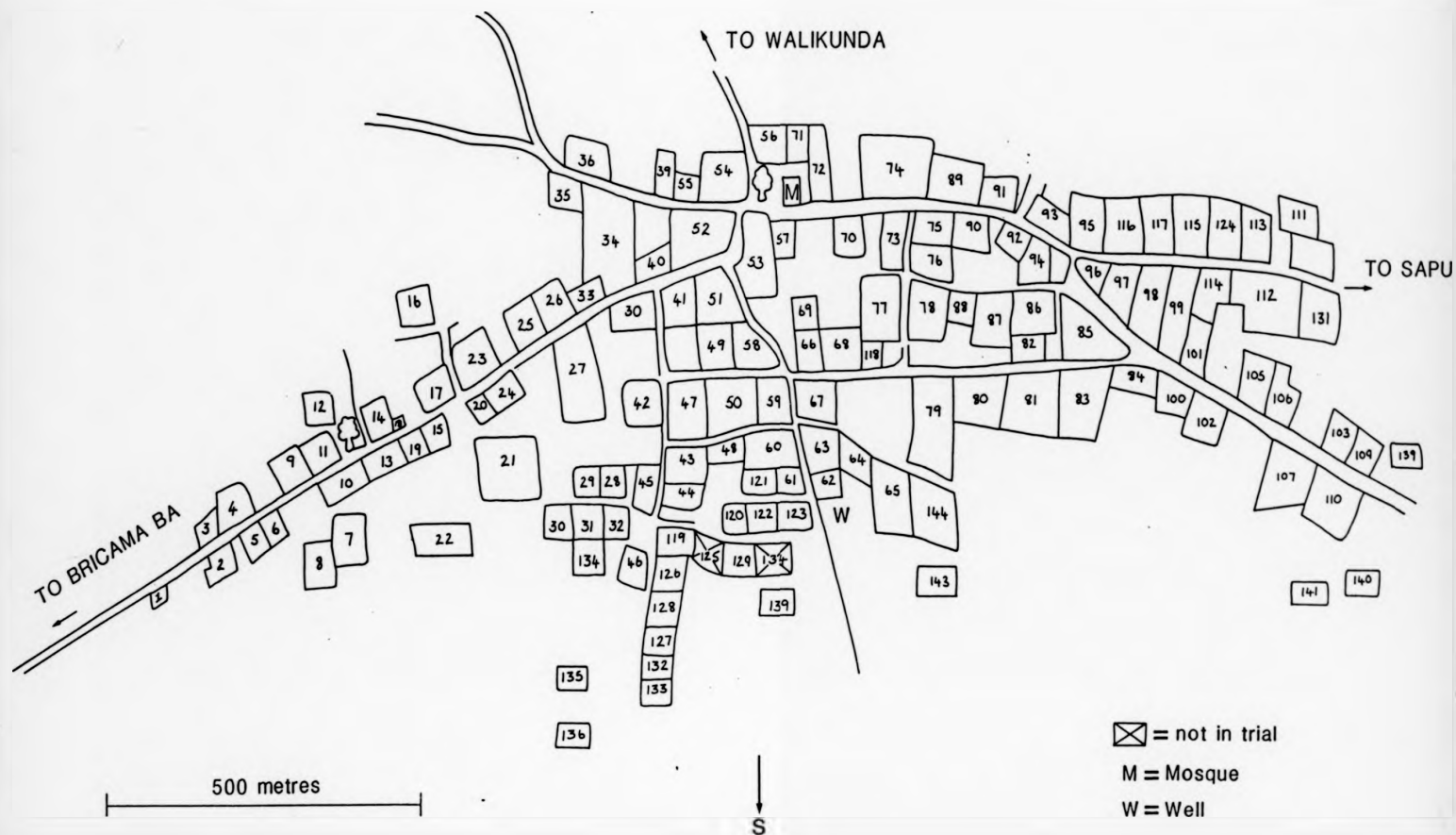


TABLE 7.1

Number of mosquitoes found weekly during bednet searches of treated nets.

Week	P	WP	L	Placebo
1	0	1 (1)	0	56 (54)
2	0	0	0	90 (88)
3	0	0	1 (1)	95 (86)
4	3 (2)	0	1 (1)	54 (47)
5	0	0	0	62 (50)
6	0	0	0	65 (63)
7	1 (1)	1 (1)	3 (1)	56 (52)
8	0	0	0	47 (44)
9	0	3 (2)	2 (1)	52 (47)
10	1 (1)	0	0	62 (38)
11	0	2 (1)	1 (0)	33 (31)
12	0	0	0	33 (29)
13	N.D			
14	0	0	5 (3)	31 (24)
15	N.D			
16	0	0	2 (1)	63 (60)

TOTAL	5 (4)	7 (5)	15 (8)	799 (713)

Number blood-fed in parentheses

N.D.- Searches not carried out.

TABLE 7.2

Regression Analyses of bioassay mortality against number of washes that the net had received

Normal Permethrin

Source of variation	d.f	SS	MS	F
Regression	1	12339.1	12339.1	29.13 ***
Residual	77	32621.6	423.657	
Total	78	44960.7	576.419	

Wash-resistant Permethrin

Regression	1	278.960	278.960	1.24 N.S
Residual	76	17140.5	225.553	
Total	77	17419.4	226.226	

Lambdacyhalothrin

Regression	1	11425.0	11425.0	39.16 ***
Residual	72	20711.9	291.717	
	72	32136.8	446.345	

*** $P < 0.001$

Figure 7.1 Mean mortalities and 95% confidence limits against number of washes for 3 pyrethroid treatments

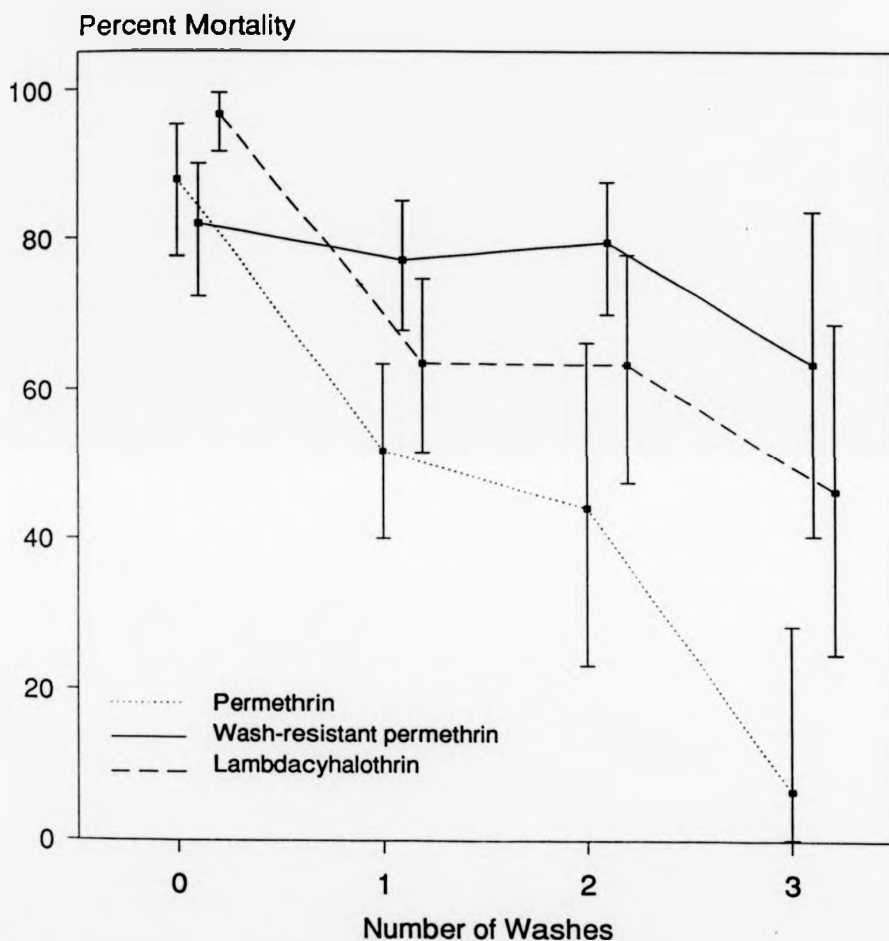


Table to show number of tests on which mean mortality is based

Treatment	Number of washes			
	0	1	2	3
Permethrin	28	37	12	2
Wash-resistant permethrin	22	27	24	5
Lamdacyhalothrin	28	23	14	8

20-30 mosquitoes per test

Figure 7.2 95% confidence limits for the regression coefficients of the three pyrethoid treatments

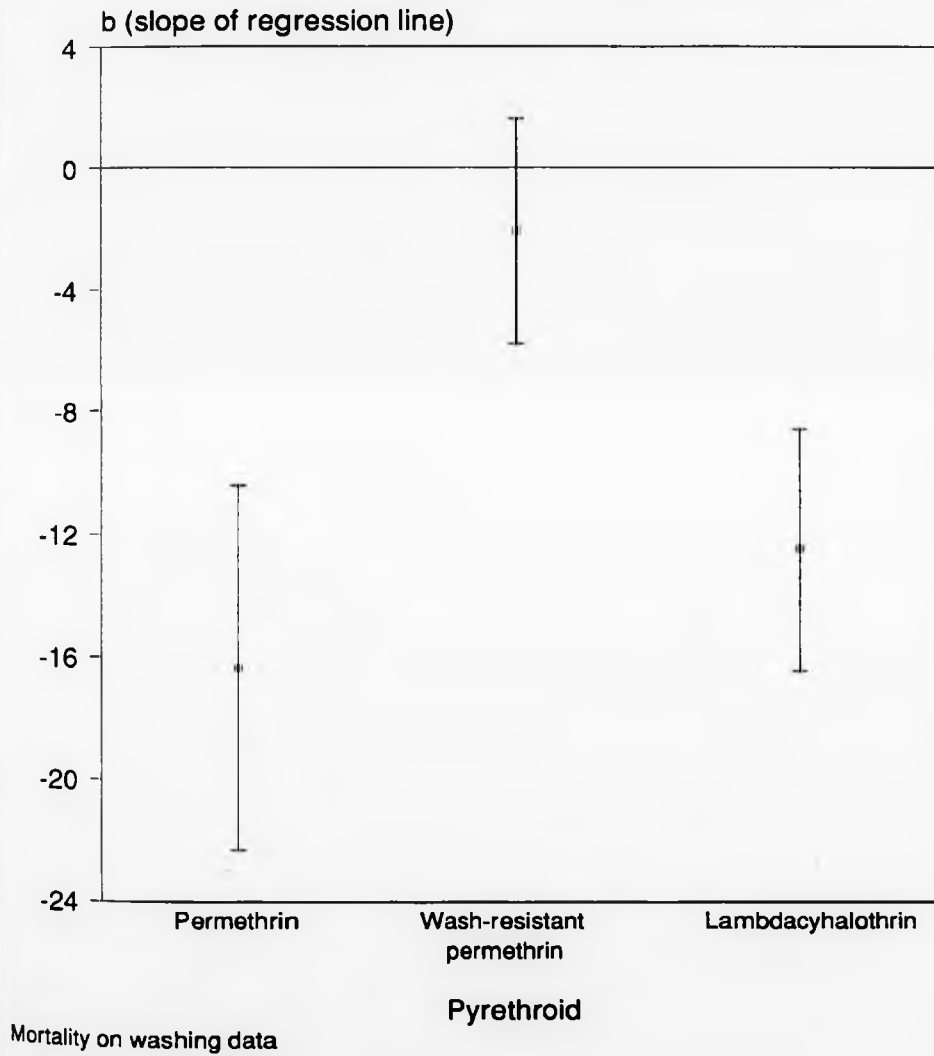


TABLE 7.3 (a)

G.C analysis of the pyrethroid uptake of 3 types of fabric commonly used for bednets in Saruja (The Gambia).

(a) Permethrin treatments.

Fabric Description	Pyrethroid/ (target deposit density, mg/m ²)	mean (n samples)	mg/m ² S.d	Range
Synthetic netting	Permethrin (500)	387 (9)	97	250-530
Synthetic sheeting (light)	"	456 (9)	210	250-860
Synthetic sheeting (heavy)	"	425 (6)	178	240-720
	TOTAL	457 (24)	163	250-860

Synthetic netting	Wash-resistant permethrin (500)	518 (9)	187	240-860
Synthetic sheeting (light)	"	447 (9)	123	270-650
Synthetic sheeting (heavy)	"	527 (6)	37	490-580
	TOTAL	493 (24)	138	240-860

TABLE 7.3 (b) G.C analysis of the pyrethroid uptake of 3 types of fabric commonly used for bednets in Saruja.

(b) Lambdacyhalothrin treatment.

Fabric Description	Pyrethroid/ (target deposit density, mg/m ²)	mean (n samples)	mg/m ² S.d	Range
Synthetic netting	Lambda-cyhalothrin (25)	17 (9)	5	12-26
Synthetic sheeting (light)	"	18 (9)	3	15-23
Synthetic sheeting (heavy)	"	32 (6)	7	22-37
	TOTAL	21 (24)	8	12-37

TABLE 7.4 Number of washes which had been received by nets in 4 treatment groups at week 16 of trial.

Treatment	Number of times nets had been washed:								n
	0	1	2	3	4	5	6	7	
Permethrin	2 (6%)	16	6	6	2	0	0	0	32
Wash-resistant Permethrin	5 (15%)	8	10	8	2	1	0	0	34
Lambda-cyhalothrin	3 (9%)	10	7	6	3	1	1	1	32
Placebo	9 (28%)	6	10	4	3	0	0	0	32

Figure 7.3 Number of washes (weighted mean)
found at monthly intervals for each treatment group

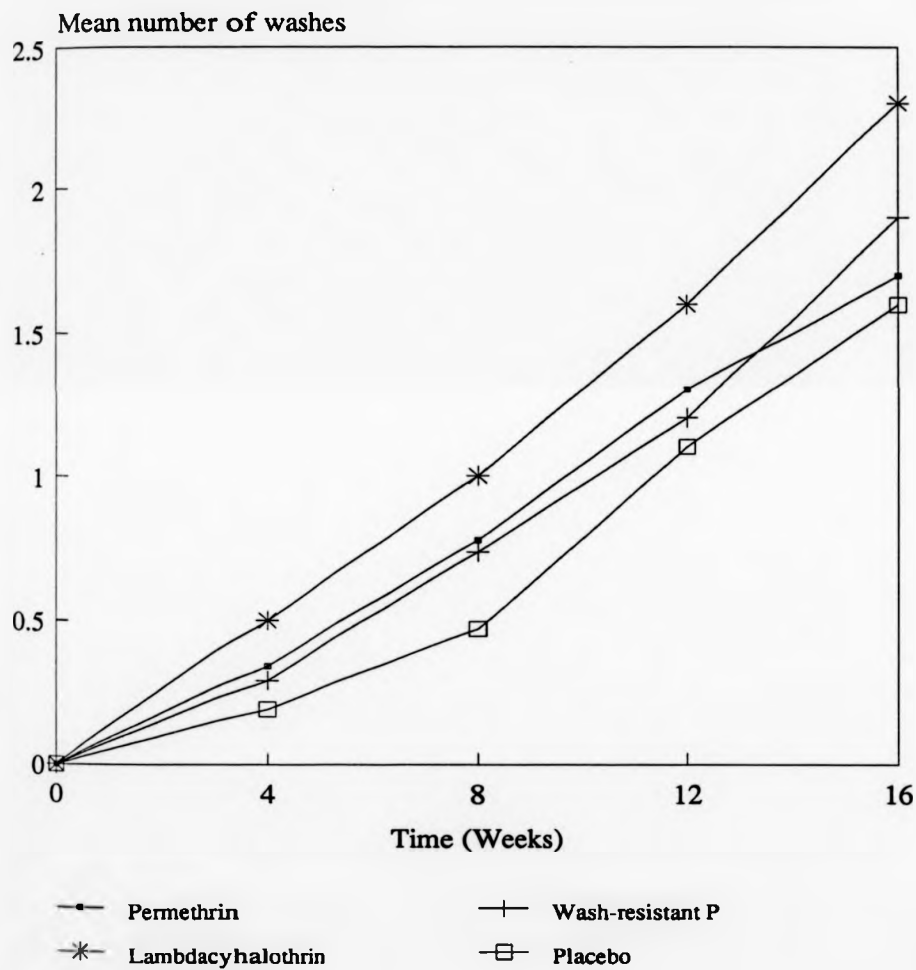


TABLE 7.5

Results of Questionnaire carried out in Saruja.

(a) GENERAL

1328 people surveyed (91% of population) unless stated.

Males = 43% Females = 57%

Use bednets = 99.4%

Why do You Use a Bednet.?

359 people surveyed

Protection from:

Mosquitoes	-	100%
Other Insects	-	49.8%
Dust	-	17.0%
Wind	-	17.0%
Rats	-	1.1%
For Security	-	0.84%
Rubbish	-	1.7%
Malaria	-	0.3%
For Privacy	-	0.3%

How many people do you share with ?:

Share bednet with no other people = 18.3%

1 other person = 41.0%

2 other people = 34.6%

3 other people = 5.6%

4 other people = 0.004%

Replace when damaged = 99.5%

Average cost = 60.00 Gambian Dalasis (= \$8.00)

Other types of protection against mosquitoes.

Churai = 37%

Coil = 52%

Nothing = 11%

Average amount spent per month = 5.00 Gambian Dalasis (= \$0.7)

Type of bed

Metal	=	37.1%
Wood	=	52.1%
Maize stalk	=	10.8%

Type of Mattress

Sacking	=	97.5%
Foam	=	2.4%
Nothing	=	0.08% (1)

TABLE 7.5 (continued) Results of Questionnaire carried out in Saruja.

(b) BEDNETS

Fabric and construction

	Synthetic	Cotton	Mixture	Total
Light Sheeting	32.6%	1.2%	13.5%	47.3%
Heavy Sheeting	12.2%	2.3%	10.1%	24.6%
Open-weave netting	23.6%	0.5%	3.9%	28.0%
Broad-mesh netting	0.08%	0	0	0.08%

Holes

Number	All sizes	Larger than end of torch battery
0	70.9%	84.5%
1 - 5	20.3%	10.6%
6 - 10	6.5%	3.8%
11 - 20	2.0%	1.1%
21 - 30	0.38%	0.15%

Washing

Weekly	=	8.0%
Fortnightly	=	64.0%
Monthly	=	28.0%
Soap	=	100%
River water	=	10%
Well Water	=	90%
Dried outside	=	100%

TABLE 7.6

What people thought about treated Nets

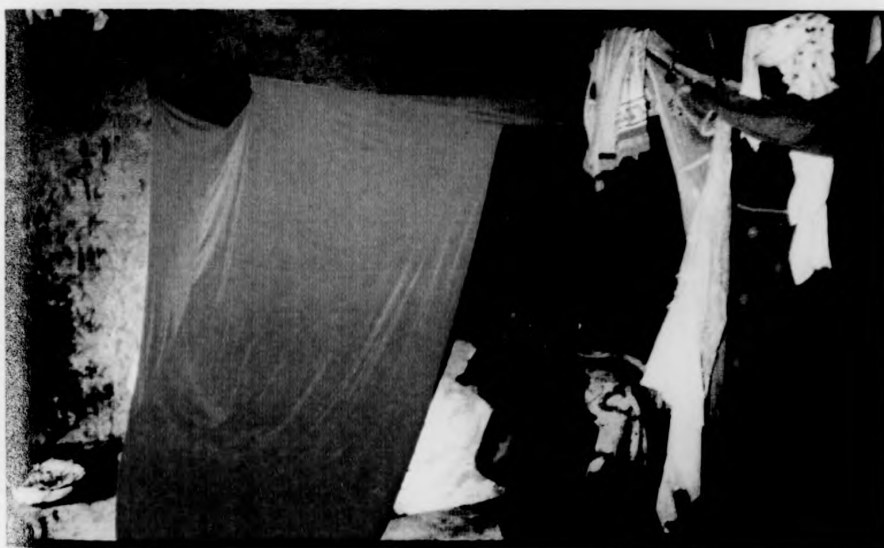
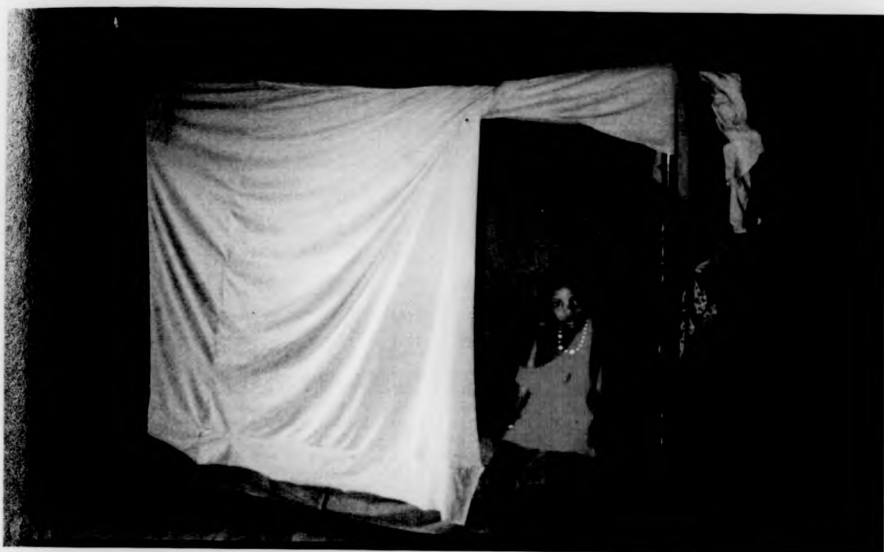
<u>LIKES</u>	TREATMENT			
	Permethrin Permethrin n=44	Wash-resistant cyhalothrin n=47 %	Lambda- n=56	Placebo n=47
Not disturbed by mosquitoes at all.	63.6	76.6	75.0	0
Disturbed by fewer mosquitoes than before.	25.0	14.9	7.1	31.9
Stops mosquitoes entering through holes.	4.5	4.3	1.8	0
Kills houseflies	2.2	0	0	0
<u>DISLIKES</u>				
Disturbed by mosquitoes	4.5	4.3	10.7	68.1
Causes Common Cold	0	0	5.4	0



Plate 7 Synthetic netting



**Plate 8
Light Synthetic sheeting**



Plates 9 (a) and (b) Heavy synthetic sheeting

7.4 DISCUSSION

After 16 weeks there was no difference between the 3 pyrethroid treatments as assessed by the number of mosquitoes found during weekly bednet searches. However, this is a fairly crude estimate of the efficacy of the nets since it gives little information on whether mosquitoes have entered the nets in the night, fed and/or been repelled. This could only be monitored by checking the nets every few hours throughout the night which would have been impractical. It is possible that video could be used to monitor whether mosquitoes were entering treated nets, feeding and then escaping because they were irritated, before the morning searches.

Bioassay data provides a more sensitive measure for assessing the relative residual toxicities of the three pyrethroids. With an exposure period of 3 minutes the wash-resistant permethrin caused the highest mortality after 3 washes. The mortality values may be considered minimum values since a hungry mosquito is likely to spend much longer searching (therefore probably coming into contact with the treatment) if it is not deterred or repelled by the insecticide. As Smith (1964) points out in his review of the origin and development of experimental hut techniques, there are 2 factors that affect the toxicity of an insecticide-treatment to mosquitoes. These are the susceptibility of the mosquito to the insecticide (i.e. the minimum dosage necessary to kill it) and the time it spends resting in contact with the insecticide. He goes on to say that it is necessary to distinguish between a mosquito's susceptibility to an insecticide and its "vulnerability" which takes into account its habits in the field. The bioassay test ignores the vulnerability of mosquitoes and tests only their susceptibility.

Toxicity of treatments is much more dependent on washing than time since impregnation - see Chapters 3 and 6 - thus the nets were grouped according to the number of washes they had received and irrespective of the week of the trial.

The wash-resistant permethrin E.C. treated nets showed little loss of insecticidal capacity after 3 washes, which was not the case for those treated with the ordinary permethrin or lambdacyhalothrin E.C.s. However, the confidence limits of

the bioassay mortality of the wash-resistant permethrin nets overlapped with those of the lambdacyhalothrin nets after 3 washes. In the experimental hut trials the wash-resistant permethrin net was never significantly better than the lambdacyhalothrin one after the 3 washes but was usually better than the permethrin one (e.g. Figure 6.2.2 (b)). The percentage of An.gambiae s.l. killed by the wash-resistant permethrin was not significantly changed by washing, and after washing it killed significantly more than the permethrin net. However, before washing, the ordinary permethrin killed a higher percentage of both An.gambiae s.l. and Mansonia spp. than the wash-resistant permethrin. This was not the case in the village trial - the confidence limits of the percent mortality due to the unwashed permethrin treated nets overlap with those of the wash-resistant formulation. There are three possible reasons for this:

- (1) The longer time period of the village trial allowed the permethrin active ingredient in the unwashed nets to fully diffuse out of the polystyrene so that the partial masking of permethrin suggested in 6.2 did not occur.
- (2) The wash-resistant permethrin formulation was made slightly differently for the village trial than the hut trial as a greater quantity was needed. The polystyrene was ground up in one solvent and this was added to other ingredients of the formulation. For the hut trial, it was made by gradually adding the polystyrene to the fully formulated permethrin E.C. This difference may have an important effect on how the a.i. was bound to the fibres.
- (3) In the village trial the villagers own nets were used. The six bioassay nets for each treatment were randomly chosen so that a range of different fibre types were bioassayed. In the hut trial, all the nets were the same fibre. The confidence limits of the mortality due to the three unwashed treatments overlapped (unlike in the hut trial) which might have been partly due to the different fibres and also because a three minute bioassay is different from the hut assessment.

The nets were generally in excellent condition, the questionnaire revealed that 71% had no holes in them at all. The population of Saruja is relatively rich and is predominantly Mandinka. The Mandinkas have the highest use of bednets of the ethnic groups in the Gambia (MacCormack, 1986). Despite this, the percentage of placebo-treated nets found with mosquitoes in them during the morning searches ranged from 22 to 59%. Most people were delighted with the pyrethroid treated nets and many of the people in the placebo treated group constantly asked for one of the better chemicals which they felt their neighbours had received. There were many requests from other people in the compounds for their nets to be dipped. An open-ended questionnaire was used to find out what people liked and disliked about their treated nets.

The two most common responses were that after pyrethroid treatment of their nets, the owners were not disturbed by mosquitoes at all or were disturbed by fewer mosquitoes than before. This questionnaire was carried out in the first month after the impregnation and it would have been interesting to have repeated it in the last month of the trial to find out whether the same responses would be evoked but, unfortunately, this was not possible. Interestingly, 32% of people sleeping under placebo treated nets responded that they were disturbed by fewer mosquitoes than before treatment. It is very unlikely that the pyrethroid treated nets could have been having a significant effect on the overall number of mosquitoes because of the small number treated. The trial was designed only to assess the amount of personal protection afforded by the treated nets (i.e. not a mass mosquito killing effect), one net per compound being used so that it was effectively an island, buffered by the rest of the compound and therefore isolated from the other treated nets. As already stated, treatment of nets was very popular. Only in two of the village compounds was treatment not given and this was because the inhabitants were away when the impregnation took place. Mosquitoes were the reason given by 100% of responders as the reason for using bednets. Thus, I feel that a large number of people using the

placebo treated nets wanted to be positive about their chemical, whilst hoping that they might get one of the better ones.

Some people also commented that the pyrethroid treatments stopped mosquitoes entering through the holes and that they killed houseflies.

Only one person of the 359 interviewed on why they used a bednet gave privacy as a response. This contrasts with MacCormack (1986) who found in a survey of 113 adults and children, 16% (only adults questioned and number not given) had purchased nets to give them privacy whilst in bed.

There were only two reasons for disliking the treated nets. One or two people in the permethrin groups were still disturbed by mosquitoes and 11% of respondents in the lambdacyhalothrin group gave this response which was possibly because these nets were less deterrent to mosquito entry into houses than the freshly treated permethrin nets. However, there was no significant difference between the groups (χ^2 test = 1.28, d.f. = 2, N.S.).

The other reason for disliking treated nets was only given by one owner of a lambdacyhalothrin net. One woman reported that the treated net had given her, and her 2 children who slept with her under the net, a "common cold". She was adamant that it was the net and reported that all the colds were gone about 3 days after the treatment. Because of this, we asked all the people sleeping under lambdacyhalothrin nets if they had got a cold after their net was treated but no one else said they had. In Tanzania with lambdacyhalothrin at 30mg/m² on nylon nets, cold like symptoms were reported by most net users for a few days after net treatment (Lines *et al.* in prep). Why some people are affected by this insecticide in this way should be further examined. The general consensus of opinion is that it occurs because of irritation of the mucosal membranes and is due more to the solvents used than the active ingredient.

Although 68% of people questioned said they washed their nets fortnightly, at the end of the 16 week trial only one of the 130 nets involved in the trial had been washed 7 times and only 10 had been washed 4 or more times. Thus it appears that

there is a tendency for people to exaggerate the frequency with which they wash their bednets.

However, in the burgeoning literature on net impregnation, The Gambia is usually quoted as a land of fortnightly bednet washers in the rainy season (McCormack and Snow, 1986; Snow *et al.*, 1987 [b]; Lindsay *et al.*, 1989 [a]; Miller *et al.*, in press), a conclusion based on questionnaires. The only way to really check this would be to have "spies" in a village, who could monitor the number of times people actually washed their nets when there was no trial or intervention study in progress.

The lambdacyhalothrin nets had been washed significantly more times than the permethrin and placebo nets. The reason for this is not clear and no indications were given that people with these nets wanted to remove the insecticide.

It would be interesting to compare the effectiveness of the treatments by bednet searches, bioassay and owner perception a year later.

The bed net search data indicate that any of the 3 pyrethroid treatments would probably be suitable for the impregnation of bednets in countries with relatively short malaria transmission seasons (e.g. The Gambia). However, further studies should be carried out to determine whether mosquitoes were entering the washed treated nets, obtaining a bloodmeal and escaping.

7.5 SUMMARY of Chapter 7

- (1) Bed net searches revealed that after 16 weeks, the numbers of mosquitoes found in placebo treated nets was far more than in nets given any of the three pyrethroid treatments. The results with these three treatments did not differ markedly from each other.
- (2) Linear regression analyses on bioassay mortality against number of washes that the nets had received, showed the permethrin formulation containing polystyrene was more wash-resistant under village conditions than an ordinary permethrin formulation or lambdacyhalothrin.

CHAPTER 8

CONCLUSIONS OF THE STUDY AND SUGGESTIONS FOR FUTURE WORK

The use of pyrethroid impregnated fibres is possibly the most important development in malaria mosquito control since the introduction of residual house spraying with DDT over 40 years ago. The alternative prospects for control of adult mosquitoes appear increasingly bleak. Among the problems faced are the emergence of both physiological and behavioural resistance by many anophelines to DDT and other insecticides (e.g. malathion) used for house-spraying, increasing refusal of householders to allow their houses to be sprayed and logistical difficulties with the delivery of insecticide etc. Resistance by Plasmodium to chloroquine is another cause for concern. We are still waiting for a malaria vaccine. Moreover, although there are vaccines for a number of infectious diseases (e.g. tuberculosis, measles, polio, tetanus, whooping cough and diphtheria), these diseases still continue to kill or cripple millions of children world-wide. In fact, when a vaccine with all the desired characteristics (cheap, long-lasting, no side-effects) is produced, the same sort of logistical problems which have hampered residual house-spraying (delivery and adequate coverage of the population) may well be the main obstacles to effective use of the vaccine.

Impregnated fabrics have the advantage of incorporating individual protection with vector control as shown in Table 8. Thus, unlike any other measure used before, they are a two pronged attack. There is also evidence (Carle et al, 1987 and Hill, unpubl) that sublethal doses of pyrethroids may affect oocyst development in the mosquito, i.e. treated netting could also be considered an antiplasmodial measure.

So, can impregnated fabrics, e.g. bednets, control malaria or even eradicate it? Firstly, it is necessary to define "control" and "eradication". The objective of control is a reduction of incidence until malaria is no longer a major public health problem.

Eradication requires a cessation of transmission and elimination of the human reservoir of infection (Bruce-Chwatt, 1980). As discussed in the Introduction, there is good evidence that in The Gambia, there have been significant reductions in malaria incidence, and the results of the large scale trial in 1989 suggest that control can be achieved. In The Gambia, transmission is seasonal and bednets are routinely used by the majority of the population so that acceptability is not a problem. Good results are also reported from China which has the benefit of hypoendemic transmission and an integrated and active control programme. Only time, and sustained and well executed bednet programmes, will tell if eradication of malaria in either countries is achievable. The problems where endemicity is perennial and at a high level are much greater (Rozendaal and Curtis, 1989).

The study on fibre/pyrethroid combinations led to the conclusion that although lambdacyhalothrin and nylon were the most insecticidal combination, the local availability of insecticide and relative cost and preferred bednet fabric should always be investigated before a final decision is made (Chapter 2).

The large differences (in comparison with the expected 10 fold differences, Herve, 1985)) in the performance of the alpha-cyano pyrethroids, compared to permethrin, highlight the importance of using the appropriate substrate (e.g. netting, mud, thatch) when testing insecticides. It should be stressed that bioassays (e.g. WHO resistance kits and plastic cones) do not include the behavioural reactions of insects to insecticide deposits. Whilst bioassays will remain the easiest way of comparing the toxicity of insecticides, wherever possible more realistic assessments e.g. using wind tunnels or small nets should also be made.

A wash-resistant formulation of permethrin was produced but it could be improved so that both a higher toxicity before washing, and more active ingredient in a form available for insect pick-up after washing, are obtained. The wash-resistant formulation of permethrin should be tested in other countries where the people have different customs regarding the washing of bednets. The wash-resistant lambdacyhalothrin formulation was not better than the normal formulation. However,

this pyrethroid caused higher mosquito mortality than the normal formulation of permethrin, after washing. As discussed in Chapter 3, it might be more appropriate to use lambdacyhalothrin for initial treatment of nets in factories because it can be more unpleasant to handle than permethrin.

The strong deterrency shown by the permethrin formulation used is a very interesting phenomenon. Further studies should be carried out to establish which constituents of the permethrin formulation are responsible. A longer study than that of the 6 weeks of the hut trials should be undertaken to measure the persistence of the deterrent effect, i.e. to find when the numbers of mosquitoes found in huts with permethrin nets equals those found in huts with untreated nets. This deterrent effect can be added to the range of effects of pyrethroids first proposed by Ruscoe (1977) and re-iterated by Herve (1982) which are shown in Figure 8.

Many parts of the study demonstrated the effect that the blank formulation had on mosquitoes stressing the importance of using the field formulations in laboratory trials, i.e. not just the a.i. in a solvent.

The difference in species susceptibility was also highlighted in both wind tunnel (Chapter 5) and hut work (Chapter 6). Recent evidence from Tanzania (Wilkes *et al.*, in prep.) has indicated that, in contrast with anophelines, village populations of Cx. quinquefasciatus were not reduced by the widespread introduction of impregnated bednets. This is surprising since Cx. quinquefasciatus bite in the evening and throughout the night and therefore would be expected to make frequent contact with treated nets. Although Cx. quinquefasciatus are known to be less susceptible than An.gambiae s.s. to permethrin on netting (Hossain and Curtis, 1989 [a]), further studies should be carried out on whether the finding is due to physiological or behavioural differences between the mosquito genera. The effect that treated fabrics have on the vectors and transmission of diseases other than malaria, e.g., filariasis, should also be examined.

Treated cotton did not kill as effectively as treated nylon in 3 minute

bioassays, or 10 minute wind tunnel experiments. In a comparison of nylon and cotton nets treated with 0.2g/m^2 permethrin, Lines *et al.*, found that there was no difference in the numbers of *An.gambiae* s.s found fed and alive. Thus, the decreased irritability shown by mosquitoes on treated cotton may make cotton as effective as nylon when overnight comparisons are made. This should be further studied. A combination of cotton and the wash-resistant permethrin may be very effective as the polystyrene may prevent the permethrin from sinking into the fibres.

The importance of monitoring resistance in mosquito populations subjected to large scale use of impregnated bednets cannot be over-emphasized. When this work was initiated, the concept of using different classes of insecticides in rotation was considered the most promising to attempt to delay the build-up of resistance. However, recent evidence (Curtis *et al.*, in press) suggests that alternation between permethrin and deltamethrin might be equally effective because deltamethrin treated netting causes higher mortality of a permethrin resistant *An. stephensi* strain than of a susceptible strain of the same species. A mixture of permethrin and an alpha-cyano pyrethroid might be even more effective, and such a combination would have the advantage that the constituents should evaporate/volatilize at about the same rates as each other.

Differences between individuals in human attractiveness to mosquitoes were demonstrated in the hut work. The reasons for these differences have never been fully elucidated and it is important that they be further examined.

The optimism of some people about possible new control strategies is tempered with caution after the setbacks of the DDT house spraying campaign. As Harrison (1978) points out, in the fight against malaria the most enduring gains have been made by gradual, social and economic as well as physical and medical changes, that together have tipped the balance in man's favour. Let us hope that judicious use of insecticide with good monitoring of resistance, ensures that impregnating fabrics continues to be a viable method of malaria control.

Table 8 Principles of comprehensive malaria control

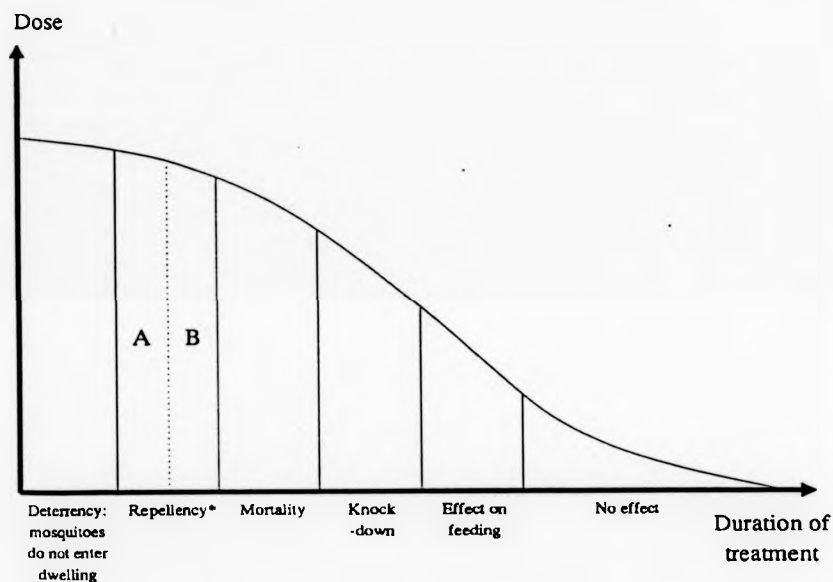
Type of control	Effect
A Individual protection *	
Mosquito repellents	Reduction of man-mosquito contact
Bednets	
House screening	
House siting	
Pyrethrum house spraying	
Coils and vaporizing mats	
B Vector Control *	
Environmental modification	Reduction of breeding sites
Chemical and biological larvicides	Reduction of vector population densities
Space spraying	"
Residual insecticide spraying	Reduction of vector longevity and population density
C Antiplasmodial measures **	
Treatment of acute cases of malaria	Elimination of malaria parasites and prevention of transmission
Prophylaxis and suppression of malaria infection	
Radical treatment of relapses	
Vaccination against malaria	

* Factors reducing the vectorial capacity

** Factors reducing the parasite reservoir

Impregnated bednets come under A, B and possibly C (see text)

Figure 8 Relationship between pyrethroid dose and mode of action (after Ruscoe, 1977 and Herve, 1982, with modifications)



*A: Repellency at a distance - mosquitoes exit without touching treated net

B: Contact repellency (excitorepellency - mosquitoes touch treated net, are irritated and exit)

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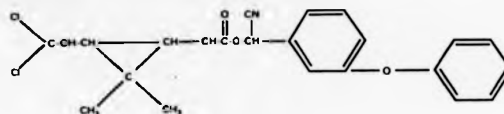
Appendix to Chapter 2

Appendix 2.1

Structures of Insecticides used:

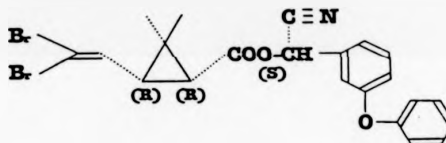
Cypermethrin

(±)-cyano-(3-phenoxyphenyl)methyl (±) cis, trans-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropane-carboxylate



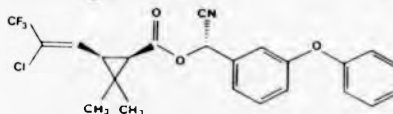
Deltamethrin

(S)-α-cyano-m-phenoxybenzyl (1R, 3R)-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane carboxylate



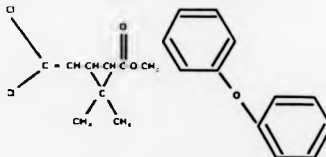
Lambdacyhalothrin

α-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate, a 1:1 mixture of the (Z)- (1R, 3R) S-ester and (Z)- (1S, 3S) R-ester



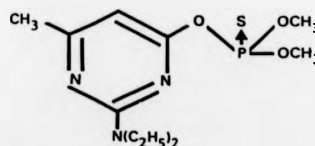
Permethrin

3-phenoxybenzyl (±) cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate



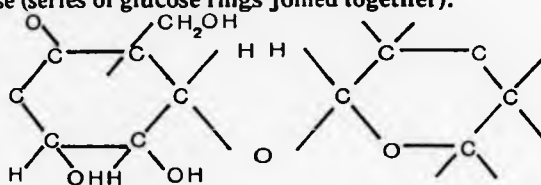
Pirimiphos-methyl

O-2-diethylamino-6-methylpyrimidin-4-yl 00-dimethyl phosphorothioate



Structure of Fibres:

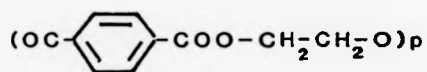
Cotton = cellulose (series of glucose rings joined together):



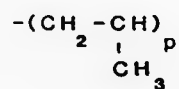
Polyamide:



Polyester:



Polypropylene:



Appendix 2.2 Mosquitoes

Anopheles gambiae

Strain G3

Colonized in London in 1975.

From MacCarthy Island, The Gambia, W.Africa.

Susceptible to all insecticides.

Rearing and Maintenance

The stock colonies of adult mosquitoes were kept in 30 cm cube cages. Each cage contained a sugar feeder (approximately 10% glucose) which was changed weekly. The stocks were fed twice a week on a guinea pig anaesthetized with Sagatal (pentobarbitone sodium B.P.). Eggs were laid two nights later in bowls lined with filter paper. When the eggs had hatched (about 2 days later, depending on temperature) approximately 200 larvae were placed in a white plastic bowl of 14" diameter, one-third full of tap water and containing about 25 g of grass and soil to encourage the growth of micro-organisms. The larva were fed on Farex baby food, once or twice a day depending on stage.

On pupating, the pupa were placed in emergence bowls, approximately 100 per enamel bowl of 6" diameter and the bowls were placed in 30 cm³ cages with 10% glucose solution feeder. Adults and larvae were maintained at $25 \pm 1^\circ\text{C}$ and 65% R.H. and 12 hour light/dark cycle.

Culex quinquefasciatus

Strain Peliyagoda

Colonized in 1988. (Selected from parental strain colonized in London in 1984 from Peliyagoda, 5km NE of Colomba, Sri Lanka).

Susceptible to organophosphates

Rearing and maintenance

The stock cage was fed once a week on a one day old chick.

The eggs were laid in bowl of water taken from larval bowl. (The glucose feeder was removed the night of laying as it was found that this encouraged oviposition). Larvae were reared in white bowls three-quarters full with tap water (approximately 200 per bowl) and fed daily on guinea food pellets.

On pupation they were treated in the same manner as the An.gambiae

Appendix 2.3 Analysis of amount of pyrethroid in treated fibres using Gas Chromatography

The following method was used. It was developed by, K. Dorgan and J. Heales (I.C.I., Jealotts Hill Research Station) for the analysis of the pyrethroid content of fibres used for bednets. It has been applied to the following active ingredients, cyfluthrin, cypermethrin, cyphenothrin, deltamethrin, fenpropathin, permethrin, d-phenothrin (sumithrin) and lambdacyhalothrin. Bednets made of cotton, nylon, polyesters (including Terylene) and combinations of all 3 have been analysed.

Summary of analytical method

1) Apparatus:

Gas chromatograph: Hewlett Packard 5880 or 5890, equipped with a split/splitless injection system operated in the split mode, or an column injection system (depending on the expected active ingredient concentration) and flame ionization detection.

Column: 25m x 0.25 mm i.d. fused silica methyl silicone CP Sil 5CB (0.1 μ m thickness) column. For on-column injection a 2 m x 0.53 mm uncoated and deactivated fused silica retention gap should be attached to the column.

Continuous roller: Luckmann "Multimix Major".

Solvents for extraction of a.i.: eg. CHCl_3 , methanol.

Internal Standards : Octacosane, octadecane.

2) Operating conditions-

Guidelines only and require optimizing with different formulations

Gas Flow Rates: All gases should be purified through molecular sieves. The carrier gas should be further purified through an oxygen trap.

Hydrogen carrier gas : $1.0 \text{ cm}^3 \text{ min}^{-1}$

Nitrogen make up gas: $30 \text{ cm}^3 \text{ min}^{-1}$

Hydrogen detector gas : $30 \text{ cm}^3 \text{ min}^{-1}$

Air : $450 \text{ cm}^3 \text{ min}^{-1}$

Split flow: approx $60 \text{ cm}^3 \text{ min}^{-1}$

Injection load : 1 mm (via an HP7673A automatic sampler)

Oven temperature:

On-column injection

Initial temperature = 50°C

Initial time = 0 minutes.

Programme rate = $30^\circ\text{C min}^{-1}$

Final temperature = 240°C .

Final time = 10 minutes.

Split injection: All pyrethroid active ingredients :

Isothermal at 200°C

(pirimiphos-methyl : isothermal at 200°C)

If pirimiphos-methyl is present with other active ingredients:

Initial temperature : 200°C

Initial time : 3 min

Programme rate : 30°C

Final value : 225°C

Final time : variable

2) Operating conditions-

Guidelines only and require optimizing with different formulations

Gas Flow Rates: All gases should be purified through molecular sieves. The carrier gas should be further purified through an oxygen trap.

Hydrogen carrier gas : $1.0 \text{ cm}^3 \text{ min}^{-1}$

Nitrogen make up gas: $30 \text{ cm}^3 \text{ min}^{-1}$

Hydrogen detector gas : $30 \text{ cm}^3 \text{ min}^{-1}$

Air : $450 \text{ cm}^3 \text{ min}^{-1}$

Split flow: approx $60 \text{ cm}^3 \text{ min}^{-1}$

Injection load : 1 mm (via an HP7673A automatic sampler)

Oven temperature:

On-column injection

Initial temperature = 50°C

Initial time = 0 minutes.

Programme rate = $30^\circ\text{C min}^{-1}$

Final temperature = 240°C .

Final time = 10 minutes.

Split injection:

All pyrethroid active ingredients :

Isothermal at 200°C

(pirimiphos-methyl : isothermal at 200°C)

If pirimiphos-methyl is present with other active ingredients:

Initial temperature : 200°C

Initial time : 3 min

Programme rate : 30°C

Final value : 225°C

Final time : variable

Injection port temperature

Split Injection 280°C

On-column injection off

Detector temperature 280°C

3) Preparation of samples

Note: the following procedure should be followed when 100 mg/m² of active ingredient (a.i.) is expected. For other concentrations the amounts should be adjusted accordingly.

Internal Standard (IS) Solution:

Octacosane should be used as the internal standard for the pyrethroids and octadecane should be used for pirimiphos-methyl.

Weigh 0.1 g of the appropriate IS into a 17 cm³ trident vial and add 10.0 cm³ CHCl₃. This is Internal Standard One (IS1).

Dilute 10 times, by taking 1.0 ml and adding 9.0 ml CHCl₃ (IS2)

Calibration Solution:

Weigh 0.01g active ingredient (i.e. permethrin, deltamethrin, lambdacyhalothrin etc.) into a trident vial. Add 1.0 ml IS2 and 9.0 ml CHCl₃. Further dilute 10 times as above.

Sample Solution:

Cut out a 10 x 10 cm square of fabric and place in a 15 ml powder bottle. Add 1.0 ml I.S.1 and 9.0 ml CHCl₃ and soak the fabric overnight with continuous rolling on the Luckman roller.

NB: A small piece of the test material should be added to CHCl₃ prior to the analysis to ensure that it does not dissolve in this solvent. If this occurs, methanol (MeOH) or another solvent should be used. (Dibutylphthalate is I.S. used with methanol).

(4) Analysis of chromatograms

Under the conditions outlined, octacosane (I.S.) elutes at around 12 minutes and octadecane at about 23 minutes. The corresponding retention times for the active ingredients analysed are as follows;

Cypermethrin	:	10.9-11.5 minutes (4 peaks)
Lambdacyhalothrin	:	6.5 minutes
Deltamethrin	:	14.75 minutes
Permethrin	:	7.8, 8.0 minutes
Pirimiphos-methyl	:	2.80 minutes

The chromatograms obtained are thus identified and analysed on the VG multichrom with Microlax II processor.

Appendix 2.4

Summary of G.C. analysis of fibres impregnated for bioassays of E.C. formulations.

FIBRE	PYRETHROID	TARGET DEPOSIT	ACTUAL DEPOSIT
		DENSITY mg/m ²	DENSITY mg/m ²
COTTON	Permethrin	3,200	3,080
		1,600	1,610
		800	740
		200	180
		50	45
	Deltamethrin	800	780
		100	110
		12.5	13
		3.125	3.0
	Lambdacyhalothrin	800	820
		100	110
		3.5	5.0
		12.5	14.0
NYLON	Permethrin	400	400
		100	80
		25	24
	Deltamethrin	12.5	15.0
		3.5	4.0
	Lambdacyhalothrin	12.5	11
		3.5	4.2
		6.5	7.0
TERYLENE	Permethrin	400	320
		100	110
		25	20
	Deltamethrin	12.5	15.0
		3.5	4.0
		12.5	11
	Lambdacyhalothrin	3.125	3.0

Appendix to Chapter 3

Emulsion stability tests are carried out to assess how well the emulsions are formed when the E.C. is added to water and how long it remains dispersed.

One ml of the test E.C. was added to 99 mls of Standard Hard Water 342 (WHO) which was maintained at 30°C. Initial "strike" and "blooming" were assessed which describe how the emulsion looks when just formed and as it disperses through the water. Top cream and separation were assessed after 30 minutes. The emulsions were assessed after two-three hours. The formulations incorporating the polymers satisfied the emulsion requirements, i.e. they did not top cream or bottom sediment but remained as heterogenous dispersions for this time period.

Appendix to Chapter 4

Calculation of amount of permethrin present in dust samples.

Sample area/calibration area x concentration of calibration
Area of calibration is 366 area units
Concentration of calibration = 0.25 ppm = 0.25 mg/1000cm³

Sample A (Room containing lambdacyhalothrin treated net):

First peak height = 45 mm
Width at half height = 2mm
Peak area = 90
Second peak height = 101 mm
Width at half height = 3
Peak area = 303
Total Area = 393 area units.
Initial sample weight = 250 mg
Final sample volume = 2.0 cm³

To calculate permethrin present:

Permethrin present = $393/366 \times 0.25 = 0.27 \text{ mg/1000 cm}^3$
 $0.27 \text{ mg} = 270 \text{ } \mu\text{g}$
 $270 \text{ } \mu\text{g/1000 cm}^3 = 0.54 \text{ } \mu\text{g/2 cm}^3$
 $\% \text{ w/w} = 0.54 \times 100/250 \times 10^{-3} = 2.16 \times 10^{-4} \% \text{ w/w}$

Sample C (untreated net):

Total peak area = 23.5 area units.
Permethrin present = $23.5/366 \times 0.25 = 0.016 \text{ mg/1000cm}^3$
 $16 \text{ } \mu\text{g/1000 cm}^3 = 0.008 \text{ } \mu\text{g/2 cm}^3$
 $\% \text{ w/w} = 0.008 \times 100/250 \times 10^{-3} = 3.2 \times 10^{-6} \% \text{ w/w}$

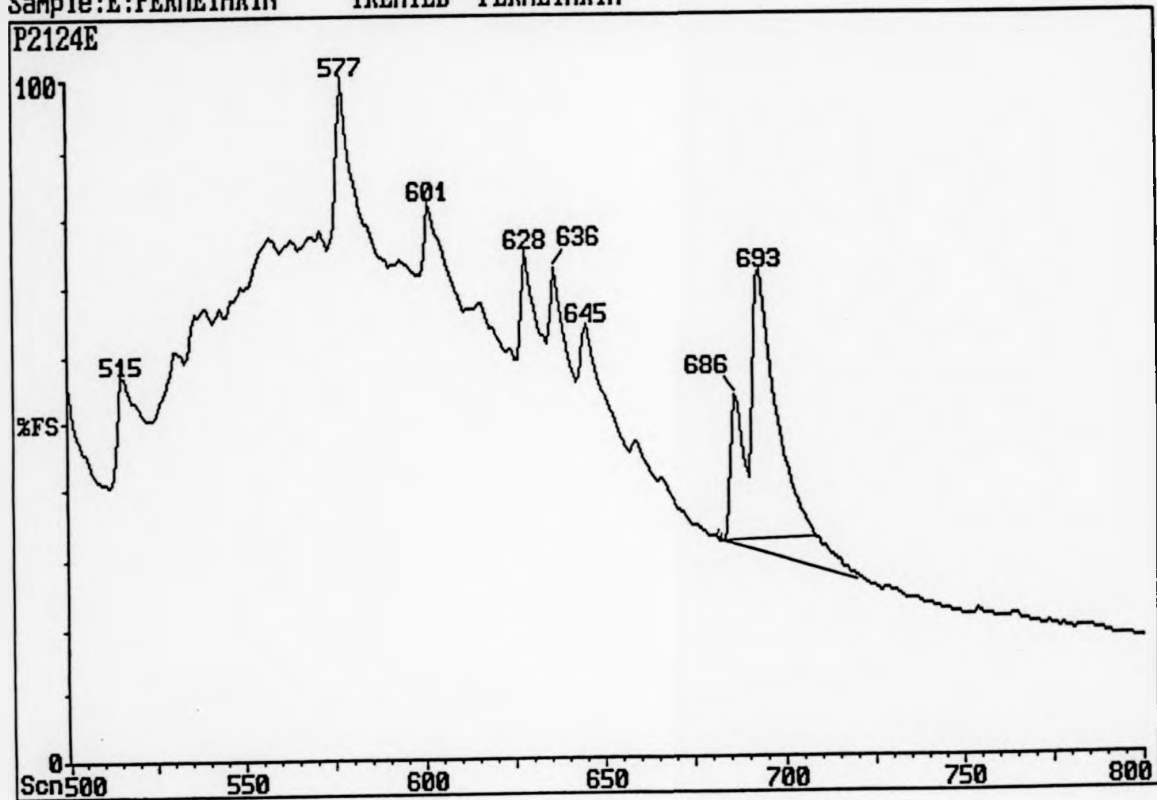
Sample E (Permethrin treated net):

Total peak area = 240
Permethrin present = $240/366 \times 0.25 = 0.16/1000 \text{ cm}^3$
 $160 \text{ } \mu\text{g/1000 cm}^3 = 0.32 \text{ } \mu\text{g/2 cm}^3$
 $\% \text{ w/w} = 0.32 \times 100/250 \times 10^{-3} = 1.28 \times 10^{-4} \% \text{ w/w}$

Chromatogram of Sample E

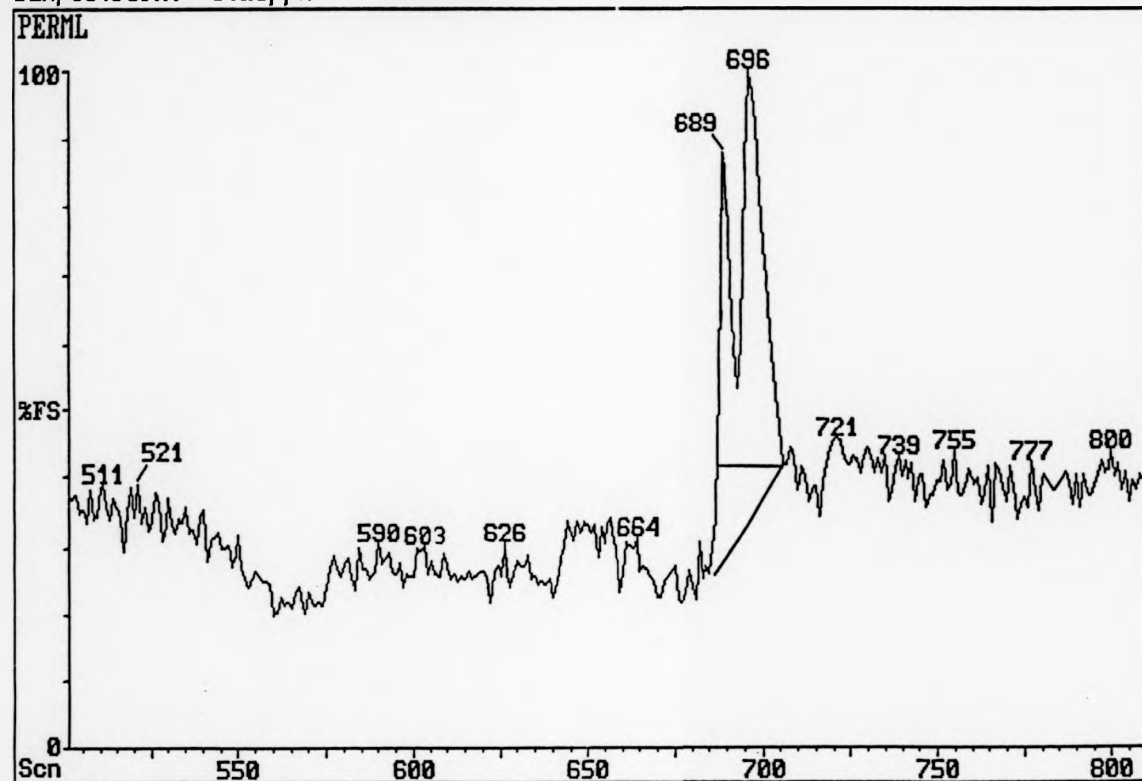
Sample:E:PERMETHRIN

TREATED PERMETHRIN



Chromatogram of Permethrin Standard

Sample: Pern. 0.25ppm 1 microlitre



Appendix to Chapter 6.

Appendix 6.1. Trial of cypermethrin, deltamethrin, lambdacyhal-

othrin, permethrin and pirimiphos-methyl.

6.1 (a) Design of Trial.

- (a) Latin square to allocate sleepers (A-F) to huts (1-6) for each week (1-6).

		HUTS					
		1	2	3	4	5	6
WEEKS	1	E	F	D	B	C	A
	2	B	A	F	D	E	C
	3	A	E	C	F	D	B
	4	D	C	A	E	B	F
	5	C	B	E	A	F	D
	6	F	D	B	C	A	E

- (b) Latin Square to allocate nets (a-f) to sleepers (A-F) and nights of the week (I-VI). The starred (*) nets represent washed or unwashed according to (c):

		SLEEPER					
		A	B	C	D	E	F
NIGHT	I	e*	b	f	d*	c*	a
	II	c	e	d*	b*	a	f*
	III	a*	f*	b	c	e	d*
	IV	f	c*	e*	a	d	b*
	V	d	a*	c*	f	b*	e
	VI	b	d	a*	e*	f*	c

- (c) Random allocation of unwashed (U)/washed(W) nets to * for each 2 weeks of the trial:

WEEK	1	2	3	4	5	6
*	U	W	U	W	W	U

- (d) Latin Square to randomize Sunday, Monday, Tuesday, Wednesday, Thursday and Friday nights to nights I-VI for each week of the study:

		WEEK					
		1	2	3	4	5	6
NIGHT	S	V	I	III	VI	II	IV
	M	II	III	V	I	IV	VI
	T	IV	VI	II	III	V	I
	W	I	II	IV	V	VI	III
	Th	III	IV	VI	II	I	V
	F	VI	V	I	IV	III	II

Appendix 6.1 (b)

ANOVA: Total number of mosquitoes found in huts and traps (ln values).

<u>Source</u>	<u>DF</u>	<u>An. gambiae</u>		<u>F</u>	<u>Mansonia</u>		<u>F</u>
		<u>SS</u>	<u>MS</u>		<u>SS</u>	<u>MS</u>	
Week	5	66.53	13.31	50.6***	16.68	3.34	18.2***
Hut	5	12.88	2.58	9.8***	6.29	1.26	6.8***
Night	5	13.83	2.77	10.5***	9.30	1.86	10.1***
Week/night♦	25	36.40	1.46	5.5***	25.78	1.03	5.6***
Sleeper	5	6.50	1.30	4.9***	4.48	0.90	4.9***
Treatment	5	10.97	2.19	8.3***	10.86	2.17	11.8***
Washing	1	0.04	0.04	0.1	3.67	3.67	20.0***
Wash/Treatment♦	5	3.14	0.63	2.4*	8.78	1.76	9.6***
Residual	159	41.80	0.26		29.218	0.18	
Total	215	192.08			115.05		

Estimates of Means:

Treatment	Unwashed	Washed	Unwashed	Washed
Untreated	3.774 (42.5)	3.428 (29.8)	3.752 (41.6)	3.414 (29.4)
Cypermethrin	3.851 (46.0)	3.791 (43.3)	3.944 (50.6)	3.794 (43.4)
Deltamethrin	3.655 (37.7)	3.537 (33.4)	3.726 (40.5)	3.678 (38.6)
Lambdacyhalothrin	3.573 (34.6)	3.760 (41.9)	3.377 (28.3)	3.946 (50.7)
Permethrin	2.884 (16.9)	3.462 (30.9)	2.652 (13.2)	3.689 (39.0)
Pirimiphos-methyl	3.889 (47.9)	3.783 (42.9)	3.296 (26.0)	3.784 (43.0)

() Back transformed means

♦ 'Interaction' effects

Approximate standard error for comparing two means = 0.18 (An. gambiae), 0.15 (Mansonia spp.)

Appendix 6.1 (b)

ANOVA: Total number of mosquitoes found dead in huts and traps (ln values).

<u>Source</u>	<u>DF</u>	<u>An. gambiae</u>			<u>Mansonia</u>		
		<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Week	5	71.27	14.25	44.3***	7.027	1.41	4.2***
Hut	5	14.76	2.95	9.2***	3.61	0.72	2.2*
Night	5	12.37	2.47	7.7***	7.94	1.59	4.8***
Week/night♦	25	34.51	1.38	4.3***	22.86	0.91	2.7***
Sleeper	5	7.22	1.44	4.5***	3.98	0.80	2.4*
Treatment	5	34.59	6.92	21.5***	38.94	7.79	23.4***
Washing	1	0.48	0.48	1.5	0.47	0.47	1.4
Wash/Treatment♦	5	3.24	0.65	2.0	4.94	0.99	3.0 **
Residual	159	51.24	0.32		52.883	0.33	
Total	215	229.67			135.62		

Estimates of Means:

Treatment	Unwashed	Washed	Unwashed	Washed
Untreated	2.918 (17.5)	2.704 (13.9)	2.452 (10.6)	2.401 (10.0)
Cypermethrin	3.775 (42.6)	3.529 (33.1)	3.819 (44.6)	3.285 (25.7)
Deltamethrin	3.564 (34.3)	3.193 (23.4)	3.598 (35.5)	3.013 (19.3)
Lambdacyhalothrin	3.476 (31.3)	3.675 (38.4)	3.420 (29.6)	3.710 (39.9)
Permethrin	2.525 (11.5)	2.967 (18.4)	2.607 (12.6)	2.972 (18.5)
Pirimiphos-methyl	3.946 (50.7)	3.547 (33.7)	3.425 (29.7)	3.373 (28.2)

() Back transformed means

♦ 'Interaction' effects

Approximate standard error for comparing two means = 0.19 (An. gambiae), 0.20 (Mansonia spp.)

Appendix 6.1 (b)

ANOVA: Percentage of mosquitoes found dead in huts and traps (arc sin \sqrt{p}).

Source	DF	<u>An. gambiae</u>			DF	<u>Mansonia</u>		
		SS	MS	F		SS	MS	F
Week	5	2.706	0.541	5.8***	5	2.53	0.506	11.4***
Hut	5	0.734	0.147	1.6	5	0.85	0.169	3.8**
Night	5	0.35	0.070	0.7	5	0.291	0.058	1.3
Week/night♦	25	2.98	0.119	1.3(*)	25	1.172	0.047	1.1(*)
Sleeper	5	0.20	0.039	0.4	5	0.05	0.010	0.2
Treatment	5	7.511	1.502	16.1*	5	6.754	1.351	30.5***
Washing	1	1.097	1.097	11.8***	1	4.738	4.738	106.8***
Wash/Treatment♦	5	0.628	0.126	1.3	5	1.601	0.320	7.2 ***
Residual	155	14.471	0.091		159	7.052	0.044	
Total	211	30.674			215	25.032		

Estimates of Means:

Treatment	Unwashed	Washed	Unwashed	Washed
Untreated	0.752 (46.7%)	0.827 (54.3%)	0.644 (36.0%)	0.729 (44.4%)
Cypermethrin	1.258 (90.5%)	1.137 (82.3%)	1.112 (80.4%)	0.910 (62.3%)
Deltamethrin	1.273 (91.4%)	1.039 (74.3%)	1.157 (83.8%)	0.820 (53.5%)
Lambdacyhalothrin	1.367 (95.9%)	1.177 (85.3%)	1.359 (95.6%)	1.029 (73.4%)
Permethrin	0.978 (68.8%)	0.914 (62.7%)	1.274 (91.4%)	0.802 (51.7%)
Pirimiphos-methyl	1.488 (99.3%)	1.169 (84.7%)	1.497 (99.5%)	0.969 (68.0%)

() Back transformed means

♦ 'Interaction' effects

Approximate standard error for comparing two means = 0.10 (An. gambiae), 0.07 (Mansonia spp.)

Appendix 6.1 (b)

ANOVA: Total number of mosquitoes found bloodfed in huts and traps (ln values).

<u>Source</u>	<u>DF</u>	<u>An. gambiae</u>			<u>Mansonia</u>		
		<u>SS</u>	<u>MS</u>	<u>F</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Week	5	71.94	14.39	19.6***	14.13	2.83	6.8***
Hut	5	3.863	0.77	1.6	5.65	1.13	2.7*
Night	5	7.424	1.48	3.1**	17.85	3.57	8.6***
Week/night♦	25	51.95	2.08	4.3***	34.97	1.40	3.4***
Sleeper	5	4.577	0.915	1.9	13.14	2.63	6.3***
Treatment	5	8.787	1.757	3.6**	28.56	5.71	13.8***
Washing	1	0.183	0.183	0.4	13.96	13.96	33.7***
Wash/Treatment♦	5	2.265	0.45	0.9	24.34	4.87	11.7***
Residual	159	77.183	0.485		65.96	0.415	
Total	215	228.18			218.55		

Estimates of Means:

<u>Treatment</u>	<u>Unwashed</u>	<u>Washed</u>	<u>Unwashed</u>	<u>Washed</u>
Untreated	2.542 (11.7)	2.327 (9.2)	2.451 (10.6)	1.574 (3.8)
Cypermethrin	2.274 (8.7)	2.063 (6.9)	1.557 (3.7)	1.672 (4.3)
Deltamethrin	2.102 (7.2)	2.281 (8.8)	1.415 (3.1)	1.675 (4.3)
Lambdacyhalothrin	1.884 (5.6)	2.331 (9.3)	0.687 (1.0)	1.882 (5.6)
Permethrin	1.704 (4.5)	1.946 (6.0)	0.216 (0.2)	1.717 (4.6)
Pirimiphos-methyl	2.438 (10.5)	2.330 (9.3)	1.465 (3.3)	2.292 (8.9)

() Back transformed means

♦ 'Interaction' effects

Approximate standard error for comparing two means = 0.24 (An. gambiae), 0.22 (Mansonia spp.)

ANOVA: Percentage of mosquitoes found bloodfed in huts and traps (arc sin \sqrt{p}).

<u>Source</u>	<u>DF</u>	<u>An. gambiae</u>			<u>DF</u>	<u>Mansonia</u>		
		<u>SS</u>	<u>MS</u>	<u>F</u>		<u>SS</u>	<u>MS</u>	<u>F</u>
Week	5	1.771	0.354	6.5***	5	0.588	0.118	5.6***
Hut	5	0.321	0.064	1.2	5	0.128	0.026	1.2
Night	5	0.117	0.023	0.4	5	0.241	0.048	2.3*
Week/night♦	25	2.82	0.113	2.1***	25	0.655	0.026	1.2*
Sleeper	5	0.988	0.198	3.6**	5	0.411	0.082	3.9**
Treatment	5	0.568	0.1136	2.1(*)	5	1.487	0.297	14.2***
Washing	1	0.0475	0.0475	0.9	1	0.238	0.238	11.4***
Wash/Treatment♦	5	0.229	0.046	0.8	5	0.712	0.142	6.8***
Residual	155	8.486	0.055		159	3.331	0.0209	
Total	211	15.345			215	7.7911		

Estimates of Means:

<u>Treatment</u>	<u>Unwashed</u>	<u>Washed</u>	<u>Unwashed</u>	<u>Washed</u>
Untreated	0.578 (29.8%)	0.714 (42.9%)	0.580 (30.0%)	0.372 (13.2%)
Cypermethrin	0.536 (26.1%)	0.444 (18.5%)	0.316 (9.7%)	0.357 (12.2%)
Deltamethrin	0.507 (23.6%)	0.607 (32.5%)	0.307 (9.1%)	0.410 (15.9%)
Lambdacyhalothrin	0.454 (19.2%)	0.536 (26.1%)	0.209 (4.3%)	0.369 (13.0%)
Permethrin	0.522 (24.9%)	0.489 (22.1%)	0.167 (2.8%)	0.377 (13.5%)
Pyrimiphos-methyl	0.562 (28.4%)	0.553 (27.6%)	0.432 (17.5%)	0.517 (24.4%)

() Back transformed means

♦ 'Interaction' effects

Approximate standard error for comparing two means = 0.08 (An. gambiae), 0.05 (Mansonia spp.)

ANOVA: Percentage of mosquitoes exiting from huts (i.e. found in exit traps and verandah traps (arc sin \sqrt{p}).

<u>Source</u>	<u>DF</u>	<u>An. gambiae</u>			<u>F</u>	<u>Mansonia</u>			<u>F</u>
		<u>SS</u>	<u>MS</u>	<u>F</u>		<u>SS</u>	<u>MS</u>	<u>F</u>	
Week	5	2.78	0.56	8.1***		0.812	0.612	6.3***	
Hut	5	0.30	0.06	0.9		0.237	0.047	1.8	
Night	5	0.32	0.06	0.9		0.249	0.050	1.9	
Week/night ♦	25	1.83	0.07	1.1		1.490	0.060	2.3***	
Sleeper	5	0.36	0.07	1.1		0.896	0.179	6.9***	
Treatment	5	0.64	0.13	1.9		1.729	0.346	13.3***	
Washing	1	0.11	0.11	1.6		0.918	0.918	35.4***	
Wash/Treatment ♦	5	0.80	0.16	2.3*		0.303	0.061	2.3*	
Residual	155	10.57	0.068			4.121	0.026		
Total	211	17.71				10.755			

Estimates of Means:

Treatment	Unwashed	Washed	Unwashed	Washed
Untreated	1.22 (88.2%)	0.98 (68.9%)	1.18 (84.5%)	1.24 (89.5%)
Cypermethrin	0.96 (67.1%)	1.15 (83.3%)	1.09 (78.6%)	1.13 (81.8%)
Deltamethrin	0.97 (68.4%)	1.08 (77.8%)	1.11 (80.2%)	1.16 (84.1%)
Lambdacyhalothrin	1.11 (80.2%)	1.06 (76.1%)	0.80 (51.5%)	1.04 (74.4%)
Permethrin	1.16 (84.1%)	1.18 (85.5%)	0.89 (60.4%)	1.15 (83.3%)
Pirimiphos-methyl	0.90 (61.4%)	1.11 (80.2%)	1.01 (71.7%)	1.16 (84.1%)

() Back transformed means

♦ 'Interaction' effects

Approximate standard error for comparing two means = 0.09 (An. gambiae), 0.06 (Mansonia spp.)

6.1 (c) Percent of occasions where mosquitoes were found alive bloodfed.

Hut-to-hut and sleeper-to-sleeper variation was not significant, so a model including night-to-night variation and the treatments was selected. Anopheles gambiae s.l.

Analysis of deviance:-

Source	Change in deviance	d.f.
Night	47.35	35
Treatment	31.34	5
Washing	6.5	1
Treatment/washing	15.4	5

Percent of occasions when live bloodfed individuals were found:

Insecticide	Unwashed	Washed
Untreated	14/19 (74)	12/17 (71)
Cypermethrin	7/18 (39)	8/18 (44)
Deltamethrin	10/18 (56)	11/18 (61)
Lambdacyhalothrin	5/17 (29) *	10/19 (53)
Permethrin	9/19 (47)	11/17 (65)
Pirimiphos-methyl	0/18 (0) ***	8/18 (44)

Mansonia spp.

Analysis of deviance:-

Source	Change in deviance	d.f.
Night	50.50	35
Treatment	36.30	5
Washing	49.0	1
Treatment/washing	26.6	5

Percent of occasions when live bloodfed individuals were found:

Insecticide	Unwashed	Washed
Untreated	18/19 (95)	15/17 (88)
Cypermethrin	11/18 (61) *	12/18 (67)
Deltamethrin	5/18 (28) ***	11/18 (61)
Lambdacyhalothrin	3/17 (18) ***	13/19 (68)
Permethrin	4/19 (21) ***	15/17 (88)
Pirimiphos-methyl	1/18 (6) ***	16/18 (89)

From X^2 comparing each treatment with the untreated net:

* = $p < 0.05$

** = $p < 0.01$

*** = $p < 0.001$

6.1 (d) Blood Meal Identification

The anti-human, anti-bovine and anti-goat/sheep plates were obtained from A. Voller, at London Zoo.

Preparation of sample

A circle of mosquito blood meal was obtained using a hole puncher. This corresponds to approximately 80 μ l of blood.

The filter paper squash was eluted for 2 hours with 1.0 ml PBS/T20 (Phosphate buffer saline containing Tween 20 : 50 ml PBS/T20 + 950 ml Distilled water).

The supernatant fluid was mixed and tested as follows:

- (1) 100 μ l of the sample was added to a well (reference samples [dilute serum 1/500 in PBS/T20 i.e. 2 μ l serum to 1.0 ml PBS/T20] were also used). The plate was incubated for 60 minutes at room temperature.
- (2) The wells were shaken out and washed 5 times with PBS/T20.
- (3) 100 μ l of the diluted conjugate was added to each well. The conjugate was diluted 1/500 in the relevant diluent before use (20 μ l in 10 ml). The plate was incubated for 60 minutes at room temperature.
- (4) The plate was washed 5 times (see 2).
- (5) 100 μ l of substrate (2 OPD tablets dissolved in 6 mls of distilled water plus 1 drop of 3% H_2O_2). The plate was incubated for 10 to 15 minutes at room temperature.
- (6) The reaction was stopped using stopping solution (2M H_2SO_4).
- (7) Each well was read visually:
Positive sample was yellow/orange
Negative sample was colourless/very pale yellow.
- (8) Samples were tested on human and bovine plates simultaneously. If they were negative on both, they were tested on a sheep/goat plate.

To detect human blood feed :

Use plate coated with anti-human IgG and anti-human IgG conjugate diluted 1/500 in anti-bovine conjugate diluent.

To detect bovine blood feed:

Use plate coated with anti-bovine IgG and anti-bovine conjugate IgG diluted 1/500 in anti-human conjugate diluent.

To detect sheep/goat blood feed :

Use plate coated with anti-sheep IgG and anti-sheep conjugate IgG diluted 1/500 in a bovine/human diluent.

6.1 (e) Cases where questionnaire on sleepers' health received positive responses

Sleepers	Net					
	U	U Washed	C Washed	L	L Washed	P
A						Stomach Pain
B	Chest pain	Headache	Headache		Chest pain	
C	Stomach pain	Stomach pain				
D				Headache		
E						Stomach pain
F						

U = Untreated
 C = cypermethrin
 L = lambda cyhalothrin
 P = permethrin

Note: in the remaining 206 cases there were no complaints

Appendix 6.2 Trial of wash-resistant formulations

6.2 (a) Design of extra week's trial to assess effect of polystyrene on mosquitoes

6 nights,
2 treatments X and Y (X = a, b, c and Y = d, e, f) i.e. 3 nets of each.
Effects of different sleepers and huts were confounded; i.e. sleeper stayed in the same hut for whole week.

	HUT	1	2	3	4	5	6
Sleeper	A	B	C	D	E	F	
NIGHT							
1	d	f	b	a	c	e	
2	b	a	d	c	e	f	
3	e	c	f	d	b	a	
4	c	e	a	f	d	b	
5	a	d	e	b	f	c	
6	f	b	c	e	a	d	

i.e. row and column design and in each hut over the 6 nights there were 3 nets with each treatment. On each night there were 3 nets with each treatment and each net was tried in each hut.

6.2 (b) ANOVA Results from comparison of untreated nets and blank plus polystyrene net.

Number* of both species (x) collected [transformed to $\ln(x + 1)$]

ANOVA				
Source of variation	SS	DF	MS	F
Night	3.429	5	0.6858	4.8 ***
Hut (= sleeper)	4.126	5	0.8252	5.8 ***
Treatment	3.702	1	3.702	26.1 ***
Residual	3.3989	24	0.1416	
Total	14.657	35		

	Adjusted means	Unadjusted means :
Untreated	4.067 (57.4)	4.753 (114.9)
Polystyrene	3.426 (29.8)	4.111 (60.0)
() back transformed	48% fewer with polystyrene.	
s.e.d. = 0.125		

t = 5.1 with 34 d.f., $p < 0.001$

* no. in hut + window traps + twice the number in the verandah traps

Mortality [$\sin^{-1} \sqrt{p}$ transformed data]

ANOVA				
Source of variation	SS	DF	MS	F
Night	0.1019	5	0.02	3.5
Hut (= sleeper)	0.1054	5	0.02	3.6*
Treatment	0.0084	1	0.0084	1.4
Residual	0.1408	24	0.0059	
<hr/>				
Total	0.3565	35		

	Adjusted means	Unadjusted means (equivalent):
Untreated	0.3113 (9%)	0.3704 (13%)
Polystyrene	0.3419 (11%)	0.4010 (15%)

() back transformed mean
s.e.d. = 0.026

t = 1.2 with 34 d.f., N.S.

% blood fed [$\sin^{-1} \sqrt{p}$ transformed data]

ANOVA				
Source of variation	SS	DF	MS	F
Night	0.1636	5	0.0327	1.9
Hut (= sleeper)	0.5302	5	0.1060	6.3***
Treatment	0.0007	1	0.0007	0
Residual	0.4049	24	0.0169	
<hr/>				
Total	1.0994	35		

	Adjusted means	Unadjusted means (equivalent):
Untreated	0.2345 (5%)	0.2680 (7%)
Polystyrene	0.2254 (5%)	0.2589 (7%)

() back transformed mean
s.e.d. = 0.043

t = 0.2 with 34 d.f., N.S.

Appendix 6.2(c)

ANOVA: Total number of mosquitoes found in huts and traps (ln values).

<u>Source</u>	<u>DF</u>	<u>An. gambiae</u>				<u>Mansonia</u>		
		<u>SS</u>	<u>MS</u>	<u>F</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Night	35	113.909	3.255	15.0***	35	100.870	2.882	12.6***
Huts	5	7.267	1.450	6.7***	5	7.424	1.485	6.5***
Sleeper	5	13.170	2.630	12.1***	5	21.960	4.392	19.2***
Treatment	11	9.217	0.838	3.9***	11	18.831	1.712	7.5***
Residual	159	34.563	0.217		159	36.439	0.229	
Total	215	178.13			215	185.53		

Estimates of Means:

<u>Treatment</u>	<u>Unwashed</u>	<u>Washed</u>	<u>Unwashed</u>	<u>Washed</u>
Untreated	2.973 (18.6)	2.760 (14.8)	2.724 (14.2)	2.619 (12.7)
Lambdacyhalothrin	2.516 (11.4)	2.736 (14.4)	2.305 (9.0)	2.665 (13.4)
Wash resistant Lambdacyhalothrin	2.404 (10.1)	2.614 (12.7)	2.181 (7.9)	2.555 (11.9)
Permethrin	2.408 (10.1)	2.624 (12.8)	2.081 (7.0)	2.519 (11.4)
Wash resistant Permethrin	2.299 (9.0)	2.571 (12.1)	1.821 (5.2)	2.263 (8.6)
Permethrin/Pyrimiphos-methyl	2.250 (8.5)	2.902 (17.2)	1.914 (5.8)	2.757 (14.7)

() Back transformed means

Approximate standard error
for comparing two means:

0.155

0.160

Appendix 6.2

ANOVA: Total mortality of mosquitoes found in huts and traps (ln values).

<u>An. gambiae</u>						<u>Mansonia</u>		
<u>Source</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>	<u>DF</u>	<u>SS</u>	<u>MS</u>	<u>F</u>
Night	35	117.50	3.357	11.1 ***	35	123.1	3.517	11.7***
Huts	5	6.487	1.297	4.3 **	5	4.556	0.9112	3.03*
Sleeper	5	12.54	2.508	8.3***	5	21.95	4.39	14.6***
Treatment	11	64.37	5.852	19.4***	1	168.72	6.25	20.8***
Residual	159	48.03	0.302		159	47.75	0.3003	
Total	215	248.92			215	266.04		

Estimates of Means:

Treatment	Unwashed	Washed	Unwashed	Washed
Untreated	0.242 (0.3)	0.259 (0.3)	0.170 (0.2)	-0.049 (0)
Lambdacyhalothrin	1.983 (6.3)	1.848 (5.3)	1.752 (4.8)	1.695 (4.4)
Wash resistant				
Lambdacyhalothrin	1.869 (5.5)	1.544 (3.7)	1.662 (4.3)	1.636 (4.1)
Permethrin	1.580 (3.9)	1.438 (3.2)	1.389 (3.0)	1.461 (3.3)
Wash resistant Permethrin	1.179 (2.3)	1.576 (3.8)	1.053 (1.9)	1.422 (3.1)
Permethrin/				
Pirimiphos-methyl	1.341 (2.8)	1.724 (4.6)	1.181 (2.3)	1.567 (3.8)

() Back transformed means
Approximate standard error
for comparing two means:

0.183

0.183

Appendix 6.2

ANOVA: Proportion of mosquitoes which entered huts that were killed ($\arcsin \sqrt{p}$).

Source	DF	<u>An. gambiae</u>			DF	<u>Mansonia</u>		
		<u>SS</u>	<u>MS</u>	<u>F</u>		<u>SS</u>	<u>MS</u>	<u>F</u>
Night	35	3.101	0.0886	1.4*	35	3.278	0.094	1.6*
Huts	5	0.742	0.148	2.3*	5	0.3769	0.075	1.3
Sleeper	5	0.556	0.111	1.7	5	0.369	0.073	1.2
Treatment	11	19.75	1.796	28.2***	11	25.77	2.343	39.4***
Residual	159	10.124	0.064		157	9.337	0.060	
Total	215	34.270			213	39.133		

Estimates of Means:

Treatment	Unwashed	Washed	Unwashed	Washed
Untreated	0.209 (4%)	0.316 (10%)	0.171 (3%)	0.156 (2%)
Lambdacyhalothrin	1.311 (93%)	0.914 (63%)	1.266 (91%)	0.804 (52%)
Wash resistant				
Lambdacyhalothrin	1.251 (90%)	0.787 (50%)	1.269 (91%)	0.910 (62%)
Permethrin	1.023 (73%)	0.712 (43%)	1.139 (82%)	0.723 (44%)
Wash resistant Permethrin	0.817 (53%)	0.886 (60%)	0.951 (66%)	0.894 (61%)
Permethrin/ Pirimiphos-methyl	0.974 (68%)	0.737 (45%)	1.030 (74%)	0.692 (41%)

() Back transformed means
Approximate standard error
for comparing two means:

0.0841

0.0813

Appendix 6.2

ANOVA: Total number of bloodfed (ln values) and proportion found bloodfed (arc sin \sqrt{p}) of An.gambiae.

<u>Source</u>	<u>DF</u>	<u>Total number</u>		<u>Proportion</u>		<u>SS</u>	<u>MS</u>	<u>F</u>
		<u>SS</u>	<u>MS</u>	<u>F</u>	<u>DF</u>			
Night	35	77.56	2.216	4.8***	35	2.740	0.078	1.9***
Huts	5	5.511	1.102	2.4*	5	0.193	0.039	0.9
Sleeper	5	23.83	4.766	10.4***	5	0.9489	0.190	4.6**
Treatment	11	38.16	3.469	7.5***	11	2.506	0.228	5.5***
Residual	159	73.192	0.460		159	6.565	0.0413	
Total	215	218.25			215	12.953		

Estimates of Means:

Treatment	Unwashed	Washed	Unwashed	Washed
Untreated	1.476 (3.4)	1.165 (2.2)	0.426 (17%)	0.375 (13%)
Lambdacyhalothrin	0.391 (0.5)	0.901 (1.5)	0.135 (2%)	0.265 (7%)
Wash resistant				
Lambdacyhalothrin	0.433 (0.5)	0.318 (0.4)	0.191 (4%)	0.119 (1%)
Permethrin	0.129 (0.1)	0.705 (1)	0.067 (0%)	0.228 (5%)
Wash resistant Permethrin	0.172 (0.2)	0.560 (0.8)	0.110 (1%)	0.227 (5%)
Permethrin/ Pirimiphos-methyl	0.021 (0)	1.039 (1.8)	0.051 (0%)	0.240 (6%)

() Back transformed means
Approximate standard error
for comparing two means:

0.226

0.068

Appendix 6.2

ANOVA: Proportion of mosquitoes exiting (i.e. number in traps /total number found) [arc sin \sqrt{p}]

Source	DF	An. gambiae			DF	Mansonia		
		SS	MS	F		SS	MS	F
Night	35	4.161	0.119	1.8**	35	3.481	0.099	3.0***
Huts	5	0.329	0.066	1.0	5	0.459	0.092	2.7*
Sleeper	5	0.375	0.075	1.1	5	0.142	0.028	0.8
Treatment	11	0.864	0.079	1.2	11	6.087	0.553	16.6***
Residual	159	10.565	0.066		157	5.249	0.033	
Total	215	16.293			213	15.418		

Estimates of Means:

Treatment	Unwashed	Washed	Unwashed	Washed
Untreated	1.231 (89%)	1.271 (91%)	1.336 (95%)	1.283 (92%)
Lambdacyhalothrin	1.102 (80%)	1.210 (88%)	0.826 (54%)	1.163 (84%)
Wash resistant				
Lambdacyhalothrin	1.150 (83%)	1.305 (93%)	0.859 (57%)	1.064 (76%)
Permethrin	1.140 (83%)	1.248 (90%)	0.881 (60%)	1.170 (85%)
Wash resistant Permethrin	1.127 (82%)	1.193 (86%)	0.917 (63%)	1.080 (78%)
Permethrin/ Pirimiphos-methyl	1.247 (90%)	1.286 (92%)	0.935 (65%)	1.272 (91%)

(%) Back transformed means

Approximate standard error
for comparing two means:

0.086

0.061

Appendix 6.3 Further analysis of the data from the experimental huts

6.3.1. Deterrency of permethrin treated net.

Numbers of An.gambiae s.l. found at start (S) and end (E) of trial with untreated net.

	S	E
x	32.2	37.0
s.d.	26.0	30.0

$s = 28.07$, where s = population standard deviation

$t = 0.296$, d.f. = 10, N.S.

Numbers of An.gambiae s.l. found at start (S) and end (E) of trial with permethrin treated net.

	S	E
x	5.83	30.83
s.d.	3.25	24.7

$s = 17.62$

$t = 2.46$, d.f. = 10, $p < 0.05$

Numbers of An.gambiae s.l. found at end of trial with untreated (u) net with numbers found at end of trial with permethrin net (p)

	E(u)	E(p)
x	37.0	30.83
s.d.	30.0	24.7

$s = 27.48$

$t = 0.39$, d.f. = 10, N.S.

Numbers of Mansonia spp. found with untreated net at start (S) and end (E) of trial.

	S	E
x	30.8	80.3
s.d.	20.5	40.9

$s = 32.4$

$t = 2.65$, d.f. = 10, $p < 0.05$

6.3.2 Mortality associated with the untreated net
 χ^2 test for trend (Kirkwood, 1988).

Data divided into weeks 1-6:

	<u>An.gambiae s.l</u>		<u>Mansonia spp</u>	
	Alive	Dead	Alive	Dead
1	181	12	173	12
2	161	2	160	9
3	99	8	117	9
4	171	20	245	33
5	237	33	251	31
6	196	26	401	81
rx =	441		930	
nx =	4286		6315	
nx ² =	19606		30845	
N =	1146		1522	
R =	101		195	
N-R =	1045		1327	

$$\chi^2_{\text{trend}} = \frac{(|A| - 0.5)^2}{B}, \text{ d.f.} = 1$$

$$\text{Where } A = \sum (rx) - \frac{R}{N} \sum (nx)$$

$$\text{and } B = \frac{R(N-R)}{N^2(N-1)} [N \sum (nx^2) - (\sum nx)^2]$$

6.3.2 Mortality associated with the untreated net
 X^2 test for trend (Kirkwood, 1988).

Data divided into weeks 1-6:

	<u>An.gambiae s.l</u>		<u>Mansonia spp</u>	
	Alive	Dead	Alive	Dead
1	181	12	173	12
2	161	2	160	9
3	99	8	117	9
4	171	20	245	33
5	237	33	251	31
6	196	26	401	81
rx =	441		930	
nx =	4286		6315	
nx ² =	19606		30845	
N =	1146		1522	
R =	101		195	
N-R =	1045		1327	

$$X^2_{\text{trend}} = \frac{(|A| - 0.5)^2}{B}, \text{ d.f.} = 1$$

$$\text{Where } A = \sum (rx) - \frac{R}{N} \sum (nx)$$

$$\text{and } B = \frac{R(N-R)}{N^2(N-1)} [N \sum (nx^2) - (\sum nx)^2]$$

7.1

Saruja Bednet Questionnaire No.1Survey number Date (DD/MM/YY) / / Age (years)

Name:

Compound: [name]..... [no.] . . . Number of people in compound Sex (M/F) Section A: ATTITUDESDo you use a bednet Y/N If yes, do you share it Y/N If so, with how many others How much did it cost When was it bought (Year) Will it be replaced when very damaged Y/N Washing netsHow often is the net washed (Weekly; Fortnightly; Monthly) How is it washed: By Hand Y/N Soap Y/N Water - River / Well Where dried - Inside / Outside hut

Other personal protection - Churai / Spray / Coil / None . 1 1 1

Other types of personal protection.....

How much spent (Dalasis per month) 1 1 1

Section B: OBSERVATIONS

What type of material

(a) Light sheeting - Synthetic/Cotton/Mixture . . . 1 1 1

(b) Heavy sheeting - Synthetic/Cotton/Mixture . . . 1 1 1

(c) Open weave netting - Synthetic/Cotton/Mixture . . 1 1 1

(d) Broadmesh netting - Synthetic/Cotton/Mixture . . 1 1 1

How many holes in the net 1 1 1

How many holes can the end of a torch battery go through 1 1 1

Type of beds - Metal/Wood/Maize Stalk. . . . 1 1 1

Mattress - None/Goat skin/Sacking/Foam . . . 1 1 1

Survey Number. 1 1 1 1 1 1

Date of Interview 1 1 1 1 1 1 1 1

Why do you use a bednet ?

(1) Mosquitoes 1 1

(2) Other insects ;

House flies. 1 1

Spiders 1 1

Ants 1 1

Bed bugs 1 1

Ticks 1 1

Not Specified 1 1

(3) Dust. 1 1

(4) Privacy. 1 1

(5) Malaria. 1 1

(6) Lizards. 1 1

(7) Rubbish from the ceiling 1 1

(8) Wind/ Warmth 1 1

(9) Rats 1 1

(10) Security 1 1

For those with a treated net

What do you like about it ?

Not disturbed by mosquitoes at all. 1 1

Disturbed by fewer mosquitoes than before 1 1

Stops mosquitoes entering through holes in net 1 1

Kills house flies. 1 1

What dont you like about it?

Disturbed by mosquitoes 1 1

It smells 1 1

It gives me common cold 1 1